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(54) Title: AGENTS AND METHODS FOR BINDING TO ELAM-1

(57) Abstract

Identification of the sialyl Lewis X determinant as the determinant responsible for ELAM-1-dependent adhesion of neutrophils to endothelium has resulted in the development of a number of agents for binding to ELAM-1 and methods for treating ELAM-1 mediated diseases.

Description

Agents and M thods for Binding to ELAM-1

Technical Field

        The present invention relates to compounds and methods
for binding to ELAM-1.

Background Art

10   The LEC-CAM/SELECTIN family of cell adhesion molecules
mediates adhesive interactions between circulating leukocytes
and the vascular endothelium (Stoolman, Cell, vol. 56, pp.
907-910 (1989); and Geng et al., Nature, vol. 343, pp. 757-760
(1990)). These molecules participate in the recruitment of
15   neutrophils and monocytes to inflammatory lesions (Carlos and
Harlan, Immunol. Rev., vol. 114, pp. 1-24 (1990); and Jutila
et al., Transplantation, vol. 48, pp. 727-731 (1989)), in the
adhesion of activated platelets to leukocytes (Larsen et al.,
Cell, vol. 59, pp. 305-312 (1989)), in the recirculation of
20   normal lymphocytes through the lymphoid system (Yednock et
al., Adv. Immunol., vol. 44, pp. 313-378 (1989)), and in the
hematogenous dissemination of lymphoid malignancies (Bartgatze
et al., J. Exp. Med., vol. 166, pp. 1125-1131 (1987)). Three
structurally related receptors have thus far been identified -
25   ELAM-1 (Bevilacqua et al., Science, vol. 243, pp. 1160-1165
(1989)), GMP-140/PADGEM (Bonfanti et al., Blood, vol. 73, pp.
1109-1112 (1989); and Johnston et al., Cell, vol. 56, pp.

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1033-1044 (1989)), and LEC-CAM1 (consensus term referring to
the antigen expressing the Mel14 epitope in the mouse (Bowen
et al., _J. Cell. Biol._, vol. 109, pp. 421-427 (1989); Lasky et
al., _Cell_, vol. 56, pp. 1045-1055 (1989)) and its human
5 homologues (Leu8/TQ1; Camerini et al., _Nature_, vol. 342, pp.
78-82 (1989); LAM, Tedder et al., _J. Exp. Med._, vol. 170, pp.
123-133 (1989); and DREG, Kishimoto et al., _Proc. Natl. Acad._
Sci., vol. 87, pp. 2244-2248 (1990))). The N-terminal domains
of these molecules are homologous to one another and to a
10 variety of calcium-dependent carbohydrate recognition domains
(CRDs) containing a structural motif originally described by
Drickamer and colleagues (Drickamer, _J. Biol. Chem._, vol. 263,
pp. 9552-9560 (1988)). The CRD of LEC-CAM1 mediates adhesion
to high endothelial venules (HEV) in vitro (Geoffroy and
15 Rosen, _J. Cell Biol._, vol. 109, pp. 2463-2470 (1989); and
Yednock and Rosen, _Adv. Immunol._, vol. 44, pp. 313-378 (1989))
and appears to initiate lymphocyte recirculation through
binding to one or more sialylated ligands on the HEV in vivo
(Rosen et al., _Science_, vol. 228, pp. 1005-1007 (1985); and
20 Rosen et al., _J. Immunol._, vol. 142, pp. 1895-1902 (1989)).

ELAM-1 mediates the adhesion of blood leukocytes to
inflammed endothelium. It is induced by a variety of soluble
mediators of inflammation including but not limited to the
immune cytokines interleukin-1 (IL-1) and tumor necrosis
25 factor (TNF), and the bacterial endotoxins (LPS). The immune
cytokines are elaborated by monocytes, keratinocytes and
possibly fibroblasts in response to inflammatory stimuli such
as infection, autoimmune response and tissue necrosis
resulting from ischemic injury. LPS refers to a heterogeneous
30 group of lipopolysaccharides derived from the cell walls of
gram negative bacteria. These broad spectrum inflammatory

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stimuli contribute to the vascular complications of bacterial sepsis including the acute respiratory distress syndrome (ARDS) and shock. The expression of ELAM-1 on the microvasculature in such diseases as psoriasis, rheumatoid

5   arthritis and delayed hypersensitivity reactions further indicates a role for this molecule in the development of pathologic inflammation. In brief, ELAM-1 dependent attachment of leukocytes to the endothelium may contribute to diverse illnesses in which the inflammatory response leads to

10 tissue damage including (1) the acute respiratory distress syndrome (ARDS); (2) rheumatoid arthritis (Grober, J. et al., submitted; Koch, A.E. 1990, _Am. J. Pathol._, in press); (3) Psoriasis and hypersensitivity disorders of the skin (Nickoloff, B. et al., submitted); (4) reperfusion injury

15 resulting from myocardial infarction and (5) hypovolemic shock.

    Accordingly, there is interest in molecules which can bind to ELAM-1 and block the attachment of leukocytes to human endothelial cells expressing ELAM-1. These molecules may be

20 used as therapeutic agents for the treatment of diseases mediated by the expression of ELAM-1 on the surface of human endothelial cells or other cells. In addition, it is desirable to enable cells, which do not normally possess the necessary determinant for recognizing ELAM-1, to express such

25 determinant and thus adhere to human endothelial cells or other cells expressing ELAM-1 on their surfaces. In this way, cells, which either naturally express or express as a result of recombinant DNA engineering a particular product, may be directed to the region of the vasculature in which the

30 endothelial cells are expressing ELAM-1, so as to specifically deliver the particular product to the stressed region of the

vasculature. Such cells could also be used as hosts for the production of recombinant glycoproteins, or other glycoconjugate-containing molecules, that contain the ligand(s) for ELAM-1, by virtue of the fact that they maintain
5 the necessary biochemical machinery necessary for the synthesis of such ligands. It is also desirable to prepare molecules in which a drug or therapeutic agent is covalently linked to a molecule which recognizes the ELAM-1 binding site and thus, are capable of directing the delivery of the drug or
10 therapeutic agent to the stressed region of the vasculature.

However, until now, the determinant on the surface of neutrophils responsible for the recognition and adhesion to ELAM-1 has remained unknown, and a process capable of generating, in quantity, high affinity ligands for ELAM-1 has
15 not been available.

Disclosure of the Invention

Accordingly, it is one object of the present invention to provide a method for treating diseases mediated by the elaboration of ELAM-1 on endothelial cells.

20 It is another object to provide a method for treating ischemia following reperfusion.

It is another object to provide a method for treating inflammation.

It is another object to provide a method for treating
25 infarction of myocardial tissue.

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It is another object to provide a method for treating Adult Respiratory Distress Syndrome (ARDS).

It is another object to provide a method for treating rheumatoid arthritis.

5   It is another object to provide a method for treating psoriasis and hypersensitivity diseases of the skin.

It is another object to provide molecules which bind to ELAM-1 and thus retard the adhesion of leukocytes to endothelial cells expressing ELAM-1.

10   It is another object to provide compounds in which a drug or therapeutic agent is covalently bonded to a molecule which binds to ELAM-1.

It is another object to provide transfected cells, which do not normally express the ability to adhere to endothelial
15   cells expressing ELAM-1, which as a result of their being transfected possess the ability to adhere to endothelial cells expressing ELAM-1.

It is another object to provide pharmaceutical compositions for treating diseases mediated by the elaboration
20   of ELAM-1 on endothelial cells.

These and other objects, which will become apparent during the following detailed description, have been achieved by the inventors' discovery that expression of a family of $\alpha(2,3)$sialylated, $\alpha(1,3)$fucosylated lactosaminoglycans on a
25   cell's surface results in ELAM-1 dependent endothelial

adhesion, and that compounds containing the sialyl Lewis X
determinant bind to ELAM-1.

Brief Description of the Drawings

     A more complete appreciation of the invention and many of
5 the attendant advantages thereof will be readily obtained as
the same become better understood by reference to the
following detailed description when considered in connection
with the accompanying drawings, wherein:

     Figure 1a illustrates the structures and biosynthesis of
10 type II-based sialyl Lewis X and Lewis X molecules. Terminal
galactose residues on neutral type II precursors may be
substituted with $\alpha(2,3)$-linked sialic acid via the action of
an $\alpha(2,3)$sialyltransferase ($\alpha(2,3)$sialyl-T, Weinstein et al.,
J. Biol. Chem., vol. 257, pp. 13845-13853 (1982)). The
15 subterminal GlcNAc residues on this sialylated precursor
molecule, or on its neutral predecessor, may then each be
substituted with $\alpha(1,3)$-linked fucose residues to form the
sialyl Lewis X (sLex) and Lewis X (Lex) determinants,
respectively. This occurs via the action of
20 $\alpha(1,3)$fucosyltransferases that can operate exclusively on
neutral precursors (e.g., Fuc-TI, Howard et al., J. Biol.
Chem., vol. 262, pp. 16830-16837 (1987)), or that operate on
both neutral and sialylated precursors (e.g., Fuc-TI, Howard
et al., J. Biol. Chem., vol. 262, pp. 16830-16837 (1987)). R
25 = glycoprotein or glycolipid moieties that may contain one or
more additional lactosamine (Galβ1,4GlcNAcβ1,3) repeat units.

     Figure 1b illustrates the structures of the type I-based
sialyl Lewis a and Lewis a determinants. The sialyl Lewis a

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(sLea) and Lewis a (Lea) molecules are thought to be
constructed from sialylated and neutral type I precursors,
respectively, by the action of α(1,4)fucosyltransferases, in a
manner strictly analogous to the biosynthesis of the type II
5   structures shown in Figure 1a (Hansson and Zopf, _J. Biol._
Chem., vol. 260, pp. 9388-9392 (1985)). R = glycoprotein or
glycolipid moieties that display the type I precursor
oligosaccharide.

     Figure 2a illustrates the binding of HL-60 cell lines to
10   human endothelial cell monolayers. HL-60 lines A and B were
tested for adherence to TNFα-activated HUVEC, using an
adhesion assay that isolates the ELAM-1-dependent component of
this interaction (Method II in Examples, vide infra). Cells
were allowed to adhere to TNFα-treated HUVEC monolayers, at 7-
15   10°C, either in the presence of the anti-ELAM-1 antibody BB11
(solid bars), or in the presence of the control antibody IgG2b
(open bars). Non-adherent cells were removed after 45
minutes; the fraction of remaining adherent cells are shown (%
HL-60 Bound), and are mean determinations, +/- standard
20   errors, representing a total of 14 (Hl-60 A) or 22 (HL-60 B)
separate determinations from five independent experiments.

     Figure 2b illustrates the results of the flow cytometry
analysis of cell surface oligosaccharide determinants. HL-60
cell lines A and B were subjected to indirect
25   immunofluorescence using the monoclonal antibodies directed
against carbohydrate determinants detailed in the inset.
Analyses were performed as described in the Examples, using a
Coulter Epics V instrument equipped with a 3-decade scale.

     Figure 3a illustrates the results of the flow cytometry

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analysis of transfected COS-1 cells. Cells were transfected
with two fucosyltransferase expression vectors (pCDM7-
α(1,3/1,4)FT, or pCDNA1-α(1,3)FT; labeled, respectively,
"α(1,3/1,4)FT", and "α(1,3)FT")), or with the control vector
5 pCDM7. Transfected cells were then subjected to flow
cytometry analysis with the monoclonal antibodies detailed in
the inset, as described in the Examples. Between 26 and 31%
of the cells transfected with pCDM7-α(1,3/1,4)FT or with
pCDNA1-α(1,3)FT expressed, respectively, all four Le
10 determinants, or Lex determinants only, relative to background
staining with anti-H antibody (data not shown). The data
presented here are the mean (linear) fluorescence intensities
of the antigen-positive population of transfected cells.

Figure 3b illustrates the adhesion of transfected COS-1
15 cells to endothelial cell monolayers. Untransfected COS-1
cells (NIL), or COS-1 cells transfected with
glycosyltransferase expression vectors or their control
vectors, were radiolabelled with ^{51}Cr, harvested, and tested
for adhesion on HUVEC monolayers using Method I, as detailed
20 in the Examples. Transfected cells were applied to either
TNFα-treated (+) or to untreated (-) HUVEC monolayers and
allowed to adhere for 20 minutes prior to washing. Plasmids
pCDM7-α(1,3/1,4)FT and pCDNA1-α(1,3)FT (labeled, respectively,
"α(1,3/1,4)FT", and "α(1,3)FT") encode distinct
25 α(1,3)fucosyltransferases as described in the Detailed
Description. The corresponding control vectors for these
plasmids (pCDM7 and pCDNA1) lack cDNA inserts. Plasmid pCDM7-
αGT encodes a murine α(1,3)galactosyltransferase (Larsen et
al., _Proc. Natl. Acad. Sci. USA_, vol. 86, pp. 8227-8231
30 (1989)). Plasmid pH3.4 encodes a human
α(1,2)fucosyltransferase (Rajan et al., _J. Biol. Chem._, vol.

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264, pp. 11158-11167 (1989)). COS-1 cells transfected with
these latter two plasmids do not express Lex, sLex, Lea, or
sLea determinants (data not shown). The adhesion of normal
neutrophils (PMN) to HUVEC within the same experiment is also
5   shown on a separately scaled ordinate. Error bars equal one
standard deviation.

Figure 3c illustrates the specific anti-ELAM-1 blocking
of adhesion of pCDM7-α(1,3/1,4)FT transfected cells to TNFα-
treated HUVEC monolayers. ^{51}Cr-labeled COS-1 cells
10   transfected with pCDM7-α(1,3/1,4)FT, or the pCDM7 control,
were assessed in the adhesion assay as outlined in Figure 3c,
except that the HUVEC monolayers had been pretreated with
antibody directed against ELAM-1 (BB11), with an isotope
control (IgG2b), or with antibodies against other endothelial-
15   expressed polypeptides (anti-VCAM1 4B9, anti-ICAM1, and anti-
HLA class 1), as described in the Examples. Error bars equal
one standard deviation.

Figure 4a illustrates the results of the flow cytometry
analysis of transfected Chinese hamster ovary cells. CHO
20   cells stably transfected with the fucosyltransferase
expression vector pCDM7-α(1,3/1,4)FT(CHO-FT) or with the
control vector pCDM7 (CHO-V) were subjected to flow cytometry
analysis with the monoclonal antibodies detailed in the figure
and as described in the Examples. The data presented here are
25   the mean fluorescence intensities of the entire population of
these transfected cells; virtually 100% of the CHO-FT cells
stain with anti-sLex and anti-Lex antibodies, but not with the
other three antibodies (data not shown). Likewise, the entire
population of CHO-V cells do not stain with any of the
30   antibodies.

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Figure 4b illustrates the time course of adhesion of CHO cells stably-transfected with pCDM7-α(1,3/1,4)FT(CHO-FT) or vector alone (CHO-V). Transfected cell lines were labeled with ^{51}Cr, harvested, and tested for adhesion on untreated (-)

5 or TNFα-treated (+) HUVEC monolayers. Non-adherent cells were removed after the times shown, and adhesion was determined by Method I as described in the Examples. Error bars equal one standard deviation.

Figure 4c illustrates the specific anti-ELAM-1 blocking

10 of adhesion of CHO-FT cells to TNFα-treated HUVEC monolayers. ^{51} Cr-labeled CHO-FT cells were subjected to adhesion for 10 minutes, as outlined in Figure 4b, except that the HUVEC monolayers had been pretreated with the anti-ELAM-1 antibody BB11, the isotype control IgG2b, or with antibodies against

15 VCAM1 and HLA class 1 molecules, at the same concentrations used in Figure 3c. Pretreatment with anti-ICAM1 fails to inhibit binding to TNFα-treated HUVEC monolayers (data not shown). Error bars equal one standard deviation.

Best Mode for Carrying Out the Invention

20 Accordingly, one aspect of the present invention relates to molecules, which bind to ELAM-1 as expressed on the surface of human endothelial cells and thus, are able to inhibit or prevent the adhesion of leukocytes to endothelial cells expressing ELAM-1 on their surface.

25 It has now been demonstrated that expression of an α(1,3/1,4)fucosyltransferase results in the appearance of ligands for ELAM-1 on the surface of transfected COS-1 and CHO

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cells. In COS-1 transfectants, the sialyl Lewis X (sLex),
Lewis X (Lex), Lewis a (Lea) and sialyl Lewis a (sLea)
oligosaccharides appeared at the surface while in CHO
transfectants, only new sLex and Lex structures were detected.
5 Thus, oligosaccharides terminating in the Lea and sLea
structures are not necessary for expression of ELAM-1
dependent cell adhesion. Moreover, ELAM-1-dependent adhesion
was not manifested either by COS-1 cells transfected with
pCDNA1-α1,3FT, or by the variant of the HL-60 cell line, when
10 expression of the Lex determinant occurs in the absence of the
sLex structure. It is thus concluded that expression of
α(1,3)fucosyltransferases capable of modifying acceptors
containing α(2,3)sialic acid-substituted lactosaminoglycans is
a critical step in the synthesis of the ligand(s) for ELAM-1.
15 Therefore, one or more members of the family of sialylated,
fucosylated lactosaminoglycans constructed by such enzymes are
identified as the ligands for the CRD of ELAM-1.

The identities of the protein or lipid molecules that
display the candidate oligosaccharide ligand(s) are as yet
20 unknown. However, it is concluded that polylactosamine-type
ganglioside precursors contained in normal myeloid cells
(Fukuda et al., J. Biol. Chem., vol. 260, pp. 1067-1082
(1985)) are not essential to the generation of ELAM-1 binding
activity, since the CHO host used for the stable transfection
25 experiments does not construct such molecules (Smith et al.,
J. Biol. Chem., vol. 265, pp. 6225-6234 (1990)). As this
observation demonstrates, the non-hematopoietic COS-1 and CHO
cells are unlikely to express unique, myeloid-specific protein
or lipid structures. Thus, it is concluded that the
30 oligosaccharide moieties determined by the transfected cDNA
are themselves sufficient to mediate ELAM-1 dependent

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adhesion.

The sLex structure and its mono- and polyfucosylated analogues are examples of such molecules. Thus, one aspect of the present invention relates to molecules which are able to
5 bind to ELAM-1 expressed on the surface of human endothelial cells and have the general formula (I):

$$NeuAc\alpha2{\rightarrow}3Gal\beta1{\rightarrow}4(Fuc\alpha1{\rightarrow}3)GlcNAc\alpha1{\rightarrow}R \qquad (I)$$
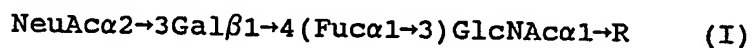
wherein NeuAc represents a sialyl group (N-acetylneuraminyl group), Gal represents a D-galactosyl group, Fuc represents a
10 L-fucosyl group, and GlcNAc represents an N-acetylglucosamine. The greek letter α indicates that the sugar moiety on the left hand side of the linkage is linked to the sugar moiety on the right hand side of the linkage by an α-linkage, and β indicates that the sugar moiety on the left hand side of the
15 linkage is linked to the sugar moiety on the right hand side of the linkage by a β-linkage. The stereochemistry of the linkages is further defined by the number and arrow system, in which the first number indicates the position of left-hand sugar moiety involved in the linkage and the second number
20 indicates the position of the right-hand sugar moiety involved in the linkage. It is to be understood that in formula (I), R is bonded directly to carbon atom number 1 of the GlcNAc group.

In formula (I), R may be OH, a protected hydroxy group, a
25 C_{1-12} alkyl group, $-O-(CH_2)_n-CO_2R''$ (wherein R'' is C_{1-4} alkyl and n is 2 to 12), $-O-(CH_2)_n-CONHNH_2$ (n is 2 to 12), $-O-(CH_2)_nCON_3$ (n is 2 to 12), a lipid or glycolipid, a glycoconjugate consisting of a serine/threonine-linked oligosaccharide on a

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protein or peptide, a glycoconjugate consisting of a free
oligosaccharide derived by enzymatic or chemical hydrolysis
from a serine/threonine-linkage to a protein or peptide, a
glycoconjugate consisting of an asparagine-linked
5   oligosaccharide on a protein or peptide, a glycoconjugate
consisting of a free oligosaccharide derived by enzymatic or
chemical hydrolysis from an asparagine linkage to a protein or
peptide. Such glycoconjugates may include polylactosamine-
type oligosaccharides whose internal GlcAc moieties may each
10 be unsubstituted, or substituted with $\alpha(1,3)$ fucose linkages.
Such molecules include single (VIM-2, Macher, B.A. et al., J.
Biol. Chem., vol. 263, pp. 10186-10191 (1988)) or multiple
(FH4 and FH5, Fukushi, Y. et al., J. Biol. Chem., vol. 259,
pp. 4681-4685 (1984); Fukushi, Y., et al., Cancer Res., vol.
15 45, pp. 3711-3717 (1985)) internal fucose residues in $\alpha(1,3)$-
linkage to internal GlcNAc moieties. All of these may be
synthesized with chemical and/or enzymatic methods, or may be
purified from natural sources (human cells of the myeloid
lineage, for example). However, that they represent part or
20 all of the oligosaccharide ligand(s) for ELAM-1 has not been
heretofore appreciated.

Suitable protecting groups for the protected hydroxy
group of R include those disclosed in European Patent
Application 184,162, which is incorporated herein by
25 reference.

In another embodiment, R may represent a drug or a label
linked to the molecule or a liposome encapsulating a drug or
label. Examples of suitable drugs include drugs encapsulated
in or otherwise incorporated into liposomes (antiinflammatory
30 agents such as corticosteroids, cytokine antagonists,

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antiinflammatory polypeptides including some interleukins,
indomethacin, gold salts, cyclosporine, and antiinflammatory
eicosanoids or enzymes that would increase the local
concentrations of antiinflammatory eicosanoids;
5 cytotoxic/chemotherapeutic agents that include adriamycin,
doxorubicin, cisplatin, vincristine, cytarabine, bleomycin,
amikacin, penicillin derivatives, Amphotericin B, or
methotrexate, for example (Ranade, V.V., _J. Clin. Pharmacol.,_
vol. 29, pp. 685-694 (1989)). Other examples wherein R may
10 represent a drug or a label linked to the molecule include
proteins, peptides, or pharmaceutical compounds, including, by
way of example, interleukins, antiinflammatory pharmaceuticals
including corticosteroids, cytokine antagonists, indomethacin,
and antiinflammatory eicosanoids or enzymes that would
15 increase the local concentrations of antiinflammatory
eicosanoids. Examples of suitable labels include drugs
encapsulated in or otherwise incorporated into liposomes
(gadolinium diethylenetriaminepentaacetic acid or other
paramagnetic chelates for magnetic resonance imaging;
20 technetium-99m and indium-111-NTA for scintigraphy; radiopaque
[iodinated or brominated compounds] for computed tomography
(Seltzer, S.E., _Radiology,_ vol. 171, pp. 19-21 (1989)).

The present compounds may be prepared by conventional
processes as described by Palcic et al, _Carbohydrate Res.,_
25 vol. 190, pp. 1-11 (1989); by Johnson et al, _Biochem. Soc._
Trans., vol. 13, pp. 1119-1120 (1985); Lemieux et al, _J. Am._
Chem. Soc., vol. 97, pp. 4056-4062, pp. 4063-4069, pp. 4069-
4075, p. 4076 (1975); Inman et al, _Immunochemistry,_ vol. 10,
p. 165 (1973); Lemieux et al, _Can. J. Biochem.,_ vol. 55
30 (1977); which are incorporated herein by reference. Thus, the
present compounds in which R is $-O-(CH_2)_nCO_2R''$ (in which R'' is

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a C_{1-4} alkyl group and n is an integer of from 2 to 12);
$-O-(CH_2)_nCONHNH_2$ (n = 2 to 12); $-O-(CH_2)_nCON_3$ (n = 2 to 12); OH;
or a C_{1-20} alkyl group may be easily prepared. The compounds
in which R is an alkoxycarbonylalkyl group may be further
5   derivatized as described by Lemieux et al, <u>J. Am. Chem. Soc.</u>,
vol. 97, pp. 4076-4083 (1975); Bundle et al, <u>J. Immunol.</u>, vol.
129, pp. 678-682 (1982); Lemieux et al, <u>Can. J. Biochem.</u>, vol.
55, pp. 507-512 (1977); and Lemieux et al, <u>Biochemistry</u>, vol.
20, pp. 199-205 (1981), which are incorporated herein by
10   reference. Thus, by this method it is possible to prepare
compounds in which R represents a group of the formula
$-O(CH_2)_nCONH-P$ in which P represents a moiety derived from a
protein, by reacting the compound in which R is $-O(CH_2)_nCON_3$
with a protein having free amino groups. Thus, in the
15   compounds having R = $-O(CH_2)_nCOHN-P$, the "NH" portion of R
originates as an amino group on the protein. Of course it is
to be understood that this reaction may proceed with multiple
additions when the protein, P, has more than one free amino
group to yield compounds with a structure
20        $(NeuAc\alpha2\rightarrow3Gal\beta1\rightarrow4(Fuc\alpha1\rightarrow3)GlcNAc\alpha1\rightarrow O(CH_2)_nCONH)_x-P$
in which x is limited by the number of free amino groups on
the protein. In addition, the present compounds in which R
includes a protein may be prepared by treating a glycoprotein
which contains a suitable substrate with the appropriate
25   enzyme, e.g., the fucosyl transferase encoded by the plasmid
pCDM7-α(1,3/1,4FT).

The fact that compounds in which R is $-O(CH_2)_nCONHNH_2$ or
$-O(CH_2)_nCON_3$ can be prepared also permits the synthesis of
compounds in which R includes a moiety derived from a drug or
30   a label. Thus, drugs or labels may be attached directly to

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the R group by conventional techniques (see: _Science_, vol. 144, p. 1344 (1964), _Immunochem._, vol. 6, p. 53 (1969); and _Peptides and Amino Acids_, Benjamin, N.Y. (1966), which are incorporated herein by reference) or via any suitable
5   bifunctional spacer. Suitable bifunctional spacers are disclosed in Haughland, _Handbook of Fluorescent Probes and Research Chemicals_, Molecular Probes, Inc., Eugene, Oregon (1989) and _Pierce Immunotechnology Catalog and Handbook_, Pierce, Rockford, Il (1990), which are incorporated herein by
10 reference.

Examples of suitable chemiluminescent labels are disclosed in German OLS No. 2,618,511, U.S. Patent 4,331,808, and Haughland, _Handbook of Fluorescent Probes and Research Chemicals_, Molecular Probes, Inc., Eugene, Oregon (1989) which
15 are incorporated herein by reference. Examples of suitable fluorescent labels are disclosed in Blecka et al, "Immunoassays in Therapeutic Drug Monitoring," Clinics in Laboratory Medicine, vol. 7, pp. 357-370 (1987) and Haughland, _Handbook of Fluorescent Probes and Research Chemicals_,
20 Molecular Probes, Inc., Eugene, Oregon (1989) which are incorporated herein by reference. Thus, the present compounds include those which may be used in chemiluminescent or fluorescent assays.

Suitable drugs include any which contain a reactive group
25 which can couple directly with either $-O-(CH_2)_n-CONHNH_2$, $-O-(CH_2)_n-CON_3$, or a terminal group of an attached bivalent spacer. In addition, the drug may be one which has been derivatized to contain a reactive group for the purpose of linking, such as those described in U.S. Patent 4,331,808,
30 incorporated herein by reference.

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In addition, the present invention relates to liposomes
which contain on their surface a liquid for ELAM-1, such as
the sialyl Lewis X determinant. The production of liposomes
is discussed in U.S. Patent Nos. 4,743,560 and 4,429,008;
5   Batzri et al, _Biochimica Biophysica Acta._, vol. 298, pp. 1015-
1019 (1973); Szoka et al, _Proc. Natl. Acad. Sci. USA_, vol. 75,
pp. 4194-4198 (1978); Kojima et al, _J. Biological Chem._, vol.
264, pp. 20159-20162 (1989); and Eggens et al, _J. Biological
Chem._, vol. 264, pp. 9476-9484 (1989); which are incorporated
10   herein by reference. Thus, the present liposomes may be
prepared by reacting liposomes which contain thiol reactive
groups (see U.S. Patent 4,429,008) with a compound to
introduce free amino groups on the surface of the liposome,
and then reacting the liposome having free amino groups on the
15   surface with a compound of formula (I) in which R is
$-O-(CH_2)_n-CON_3$. For example, a liposome having maleimide
groups on its surface may be reacted with a compound such as
$H_2NCH_2CH_2SH$, to obtain a liposome having free $-NH_2$ groups.
Alternatively, the present liposomes may be prepared by
20   incorporating a molecule such as a ceramide which is bonded,
either directly or through a suitable bifunctional spacer, to
a ligand of ELAM-1, such as the sialyl Lewis X determinant
(see: Eggens et al, _J. Biological Chem._, vol. 264, pp. 9476-
9484 (1989); Batzri et al _Biochim. Biophys. Acta._, vol. 298,
25   pp. 1015-1019 (1973); and Szoka et al, _Proc. Natl. Acad. Sci._,
vol. 75, pp. 4194-4198 (1978)). The present liposomes may
also contain drugs or other molecules as described in U.S.
Patent Nos. 3,993,754 and 4,263,428, which are incorporated
herein by reference. Additionally, the present liposomes may
30   also encapsulate labels for NMR imaging or radionucleotide
scanning. Suitable labels for use as radioactive diagnostics
are disclosed in U.S. Patent 4,094,965 and Canadian Patent

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Application 946,741, which are incorporated herein by
reference. Suitable labels for NMR imaging are disclosed in
French Patent Application 2,612,400, _Eur. J. Med._, vol. 24,
pp. 241-247 (1989), UK Patent Application 2,214,507, and
5 _Radiology_, vol. 175, pp. 483-488 (1990), which are
incorporated herein by reference.

Another aspect of the present invention relates to
transfected cells, which do not normally exhibit ELAM-1-
dependent adhesion to endothelial cells, but which as a result
10 of their transfection exhibit ELAM-1-dependent adherence to
endothelial cells and a method for the production of such
cells. Thus, such cells are those which either naturally or
as a result of transfection produce a suitable substrate and
have been transfected with either cDNA or genomic DNA encoding
15 an enzyme capable of converting said suitable substrate into
the sLex determinant.

Accordingly, the process for preparing the present cells
involves:

    (i) transfecting a starting cell, which does not express
20 the sLex determinant and which does produce a substrate, with
a DNA sequence coding for an enzyme which has the activity of
converting the substrate to a molecule which contains the sLex
determinant.

    Thus, the starting cells may be any which do not express
25 the sLex determinant but do produce a substrate which may be
converted to a molecule containing the sLex determinant, by
the enzyme encoded by the heterologous DNA which is introduced
into the cells. Examples of suitable starting cells include

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COS-1 and Chinese hamster ovary (CHO) line Ade⁻ C. The fact
that pCDM7-α(1,3/1,4)FT transfection induces expression of
ligands for ELAM-1 on non-hematopoietic cells indicates
generalized distribution of suitable "acceptor" molecules for
5   the α(1,3/1/4)FT. Therefore, it is reasonable to conclude
that stable transfection of α(1,3/1,4)FT cDNA into (and
expression in) a variety of normal cells and cell lines will
result in ELAM-1 binding activity. Suitable examples of the
enzyme include the fucosyltransferase encoded by pCDM7-
10 α(1,3/1,4FT). The plasmid pCDM7-α(1,3/1,4FT) has been
deposited in the E. coli strain MC 1069/P3 with the American
Type Culture Collection, 12301 Parklawn Drive, Rockville, MD
20852 and has been given the accession number 68448. Other
examples include an α(1,3)fucosyltransferase capable of
15 constructing Fucα(1,3)GlcNAc linkages on α(2,3)sialylated
lactosamine molecules, of the type that is expressed in human
myeloid cells (Potvin et al., J. Biol. Chem., vol. 265, 1615-
1622 (1990)) or in other species (Howard et al., J. Biol.
Chem., vol. 262, pp. 16830-16837 (1987).

20   The fucosyltransferase encoded by pCDM7-α(1,3/1,4FT) is
preferred and represents the product of the human Lewis blood
group locus (Kukowska-Latallo et al., Genes Dev., vol. 4, pp.
1288-1303 (1990)). It exhibits a uniquely broad spectrum of
acceptor substrate requirements that encompasses the
25 requirements exhibited by members of two other general classes
of α(1,3)fucosyltransferases (Prieels et al., J. Biol. Chem.,
vol. 256, pp. 10456-10463 (1981); and Palcic et al., Carbohyd.
Res., vol. 190, pp. 1-11 (1989)). Expression of this enzyme
is thought to be restricted to secretory epithelium, except in
30 Lewis blood group negative individuals who inherit an
inability to express this enzyme in any tissue (Watkins, Adv.

Human Genet., vol. 10, pp. 1-116 (1989)). This enzyme is thus probably not responsible for synthesis of the ELAM-1 ligand(s) in the myeloid lineage. The $\alpha(1,3)$fucosyltransferase encoded by pCDNA1-$\alpha(1,3)$FT is likewise not responsible since it is

5　representative of a second distinct class of $\alpha(1,3)$fucosyltransferases (Howard et al., _J. Biol. Chem._, vol. 262, pp. 16830-16837 (1987)) that are unable to utilize $\alpha(2,3)$sialic acid-substituted lactosamine molecules that are the apparent precursors for the ELAM-1 ligand(s). It

10　therefore seems likely that a member of a third class of $\alpha(1,3)$fucosyltransferases, capable of constructing the Lex and sLex moieties but not the Lea isomers (Potvin et al., _J. Biol. Chem._, vol. 265, pp. 1615-1622 (1990)), represents the enzyme that determines expression of the ELAM-1 ligand(s) in myeloid

15　cells.

When the starting cells are transfected with pCDM7-$\alpha(1,3/1,4$FT), suitable starting cells are those that produce substrates which contain carbohydrate moieties corresponding to the sialylated type II precursor shown in Figure 1a:

20　　　　　　NeuAcα2→3Galβ1→4GlcNAcα1→R

In a preferred embodiment, the starting cells are immune effector cells whose cell surface oligosaccharide structure does not allow them to be targeted to endothelium expressing ELAM-1. Thus, the present process results in such cells

25　expressing the sLex determinant and being targeted to the areas of inflammation at sites of pathology, where their immune effector functions are therapeutically beneficial. Examples of suitable immune effector cells include lymphokine-activated killer cells (LAK cells) and tumor infiltrating

lymphocytes (TIL cells) in the treatment of advanced cancer,
which produce substrates which may be converted to molecules
containing the sLex determinant by the enzymes:

(1) a Galβ1->3(4)GlcNAc α(2,3)sialyltransferase (of the
5 type described in Weinstein, J., et al., J. Biol. Chem., 257,
13845-13853 (1982) or the type that operates on O-linked
glycoconjugates, as described in Palcic et al., Carbohyd.
Res., vol. 190, pp. 1-11 (1989), and

(2) An α(1,3)fucosyltransferase capable of constructing
10 Fucα(1,3)GlcNAc linkages on α(2,3)sialylated lactosamine
molecules, of the type described in this application (encoded
by pCDM7-α(1,3/1,4)FT, and also described in Palcic et al.,
Carbohyd. Res., vol. 190, pp. 1-11 (1989) and in Kukowska-
Latallo, J.F., et al., Genes Dev., vol. 4, pp. 1288-1303
15 (1990)), or of the type that is expressed in human myeloid
cells (Potvin et al., J. Biol. Chem., vol. 265, 1615-1622
(1990)) or in other species (Howard et al., J. Biol. Chem.,
vol. 262, pp. 16830-16837 (1987).

It is to be understood that the starting cells may also
20 be transfected with another heterologous DNA sequence which
encodes for a desired polypeptide. Examples of such
polypeptides which may be expressed by heterologous DNA
sequences include, e.g., EPO, insulin, and interleukin 2.

In another embodiment, the present invention provides a
25 method for targeting a glycoprotein to cells expressing ELAM-
1. Thus, a cell which produces a first glycoprotein which can
serve as a substrate for an enzyme which will convert the
first glycoprotein to a second glycoprotein having a ligand

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for ELAM-1 will produce the second glycoprotein after transfection with a suitable enzyme, e.g., the fucosyl transferase encoded by the plasmid pCDM7-α(1,3/1,4FT). Of course, it is to be understood that the cell may produce the

5 first glycoprotein either naturally or as a result of genetic engineering. In another embodiment, the cell may be transfected to produce a second enzyme or a series of enzymes which will convert a first protein or glycoprotein, which is not directly convertable to a glycoprotein having the sialyl

10 Lewis X determinant, to a glycoprotein which can serve as substrate for an enzyme which will convert it to a glycoprotein having the sialyl Lewis X determinant. Thus, the present invention provides cells which produce glycoproteins which are targeted to ELAM-1 and glycoproteins which are

15 targeted to ELAM-1.

Of course the transfection of the starting cells with the DNA sequence encoding for the enzyme responsible for converting the substrate into the ligands for ELAM-1 and any other desired heterologous DNA sequences may be accomplished

20 by any conventional transfection technique. Suitable methods are discussed in Davis et al, Basic Methods in Molecular Biology, Elsevier Publishing Co., NY, NY (1986).

In another embodiment, the carbohydrate ligands for ELAM-1 are linked to recombinant polypeptide antiinflammatory

25 molecules vector by expressing such recombinant proteins, in a form suitable to be expressed with N- and/or O-linked oligosaccharides, in an eukaryotic host that also expresses the enzymes that construct the oligosaccharide ligand(s) for ELAM-1 (i.e., CHO-FT cells, for example).

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In another embodiment, the present cells are those which express a substrate on their surface which may be converted to the ligands for ELAM-1 through direct enzymatic action of soluble or immobilized recombinant α(1,3/1,4)FT or its

5 analogous myelod specific enzyme. Thus, the present cells may be produced by a process involving treating a cell with an enzyme which is capable of converting a determinant found on the surface of the cell into a ligand for ELAM-1. It is also possible to treat an oligosaccharide precursor by itself or on

10 another molecule with a recombinant enzyme to form an ELAM-1 ligand.

Thus, in the above-described method, the starting cells need not be transfected.

The present invention also relates to targeting of

15 therapeutics to endothelium expressing ELAM-1 through the construction and linkage of type II structures to liposomes, macromolecular carriers and single molecules. The identification of alpha 1,3 fucosyl transferases as the rate limiting step in the synthesis of ELAM-1 specific ligands, and

20 the demonstration that a cloned alpha 1,3/1,4 fucosyl transferase can generate ligands from type II precursors in a stable transfection system provides both the knowledge and the means to synthesize targeted therapeutics. Thus antiinflammatory agents with systemic toxicity, such as

25 glucocorticoids, methotrexate, cyclosporin and gold salts, could be delivered in high concentration directly to sites of active inflammation.

It is also to be understood that, as in the case of the present compounds of formula (I), the present cells and

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liposomes may contain a label suitable for use in an imaging
technique in addition to or in replacement of a drug or
therapeutic agent. In this way it is possible to target the
label to the activated endothelium, and an _in vitro_ imaging
5 technique, such as NMR or radionucleotide scanning, may be
used to specifically locate the affected area.

    The present results suggest that a leukocyte adhesion
deficiency-like phenotype (Anderson and Springer, <u>Ann. Rev.
Med.</u>, vol. 38, pp. 175-194 (1987)) occurs in individuals with
10 a genetic defect in the ability to express the appropriate
$\alpha(1,3)$fucosyltransferase in the myeloid lineage. In this
context, it is noted that many patients with adenocarcinoma,
but not healthy individuals, maintain circulating mucin-like
molecules containing sialyl Lex Moieties (Kannagi et al.,
15 <u>Cancer Res.</u>, vol. 46, pp. 2619-2626 (1986)). Such molecules
may be shed from malignant tumors in part as a consequence of
the "aberrant" expression of the sialyl Lex determinant
frequently seen in association with malignant transformation
(Fukushi et al., <u>J. Biol. Chem.</u>, vol. 259, pp. 4681-4685
20 (1984); Fukushima et al., <u>Cancer Res.</u>, vol. 44, pp. 5279-5285
(1984); and Kannagi et al., <u>Cancer Res.</u>, vol. 46, pp. 2619-
2626 (1986)). Such molecules may participate in specific
interactions with ELAM-1 acting to inhibit normal interactions
between leukocytes and the vascular wall, and thereby act to
25 blunt inflammation-induced leukocyte recruitment in these
patients. Adenocarcinoma cells have also been shown to
exhibit ELAM-1-dependent HUVEC adhesion (Rice and Bevilacqua,
<u>Science</u>, vol. 246, pp. 1303-1306 (1989)).

    The extensive homology between the CRDs of the LEC-
30 CAM/SELECTIN family raises the possibility that the endogenous

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ligands for GMP140/PADGEM and LEC-CAM1 are structurally
related to the sLex family of oligosaccharides. The former
receptor, in particular, appears to interact with the same
spectrum of normal cells and cell lines which adhere to ELAM-1
5   (Larsen et al. Cell, vol. 59, pp. 305-312 (1989)). Rosen and
colleagues have demonstrated that terminally-linked sialic
acid is a part of the vascular ligand for $gp90^{MEL14}$ (Yednock
and Rosen, Adv. Immunol., vol. 44, pp. 313-378 (1989)). Of
potential interest with respect to a fucosylated ligand for
10 this molecule are studies implicating selective inhibition of
functional activity with L-fucose, and fucoidan, a
heteropolysaccharide rich in fucose-4-sulphate (Stoolman and
Rosen, J. Cell Biol., vol. 96, pp. 722-729 (1983); and
Stoolman, Tenforde, and Rosen, J. Cell Biol., vol. 99, pp.
15 1535-1540 (1984)). In recent studies, a direct interaction
between two fucose-sulphate containing polysaccharides,
fucoidan and the egg-jelly fucan, and purified $gp90^{MEL14}$ has
been convincingly demonstrated (Yasuyuki et al., 1990). These
synthetic ligands may mimic key structural features or the
20 charge distribution of an endogenous fucosylated sialyl-
lactosamine.

In another aspect, the present invention relates to a
method of treating a disease mediated by the expression of
ELAM-1 by endothelium. Such diseases include, e.g., adult
25 respiratory distress syndrome, vasculitis, and myocardial
infarction.

In one embodiment, the treatment of the disease involves
administering an effective amount of the compound of the
formula (I). When R is not a moiety derived from a drug or
30 therapeutic agent, the compound of formula (I) binds to the

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ELAM-1 on the surface of the affected endothelial cells and
acts to block the adhesion of the neutrophils. When R is a
moiety derived from a drug or represents a drug attached to
the sLex determinant via a linking molecule, the compound also
5   acts to specifically deliver the drug to the affected area, in
addition retarding the adhesion of the neutrophils to the
endothelium.

     In another embodiment, the present treatment involves
administering cells or liposomes which have been treated
10   according to the present invention so that they carry the
ligand(s) for ELAM-1. As in the case of the compounds of
formula (I), it is possible to use the present cells and
liposomes which carry the carbohydrate ligands on their
surfaces as blocking agents. However, it is preferred that
15   the cells and liposomes used in the present treatment contain
a drug effective for the treatment of the affected area, in
addition to carrying the carbohydrate ligand(s) on their
surfaces.

     Regardless of whether the present compound, cells or
20   liposomes are being used for the treatment of a disease or the
imaging of the affected area, all and any of these may be
administered by any conventional means and in any conventional
form suitable for placing the compound, cell or liposome in
the vasculature. Preferably, the compounds, cells, and
25   liposomes are administered by injection in a form suitable
therefore, such as a solution or suspension. Such forms are
well known in the art.

     The exact dosage of the compound, cells, and liposomes
will depend on whether the compound, cells, or liposomes are

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being used as a blocking agent or to target drug or label delivery, the identity of the drug or label being delivered, if any, and the size and health of the patient. For any of the above-described purposes or cells, compounds or liposomes,

5   the exact dosage to be administered may be easily determined by one of ordinary skill.

Other features of the invention will become apparent in the course of the following descriptions of exemplary embodiments which are given for illustration of the invention

10 and are not intended to be limiting thereof.

Examples

Methods:

Cell Culture

Human umbilical vein endothelial cells were isolated and

15 propagated according to the method of Jaffe (Jaffe, in Biology of Endothelial Cells, E.A. Jaffe, Ed., Martinus Nijhoff, The Hague, pp. 1-13 (1984)). Cells were maintained in medium 199 with 20% fetal bovine serum, 100 μg/ml endothelial growth supplement (Collaborative Research), and 100 μg/ml bovine lung

20 heparin (Sigma). Cells were used between passage 2 and 4. Endothelial identity was confirmed by the presence of typical morphology at confluence, by immunofluorescence with antibody to von Willebrand factor, and by uptake of acetylated low density lipoprotein. For adhesion assays, HUVEC were passaged

25 with trypsin/EDTA (Gibco), and plated in 96 well plates (Dynatech, Method I; Falcon, Method II: see below under "HUVEC Adhesion Assays"). Cells were allowed to grow to confluence for at least two days prior to use in adhesion assays. HL-60

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lines in spinner culture were maintained between 2.5-15 x 10^6
cells/ml in RPMI 1640 supplemented with 10% fetal calf serum
and antibiotics. The Chinese hamster ovary (CHO) line Ade-C
(Oates and Patterson, Somatic Cell Genet., vol. 3, pp. 561-577
5   (1977), and Van Keuren et al., Am. J. Human Genet., vol. 38,
pp. 793-804 (1986)) was grown in α-modified Eagle's medium
supplemented with 10% fetal calf serum. Transfected CHO cells
were grown in media supplemented with G418 (Gibco) at 400
μg/ml (active drug). COS-1 cells were grown in Dulbecco's
10   modified Eagle's medium containing 10% fetal calf serum.

Glycosyltransferase Expression Vectors

        Previous publications describe the plasmids pCDM7 and
pCDM7-αGT (Larsen et al., Proc. Natl. Acad. Sci. USA, vol. 86,
pp. 8227-8231 (1989)), pCDM7-α(1,3/1,4)FT (Kukowska-Latallo et
15   al., Genes Dev., vol. 4, pp. 1288-1303 (1990)), and pH3.4
(Rajan et al., J. Biol. Chem., vol. 264, pp. 11158-11167
(1989)). Plasmid pCDNA1-α(1,3)FT contains a 3.6 kb Pstl human
genomic DNA restriction fragment that encodes an
α(1,3)fucosyltransferase (Lowe et al., unpublished). This
20   fragment was isolated from a human lambda phage rescued from a
genomic DNA library probed at low stringency with the insert
in pCDM7-α(1,3/1,4)FT. The gene segment is cloned in the
appropriate transcriptional orientation into the mammalian
expression vector pCDNA1 (InVitrogen).

25   Antibodies

        The anti-Le^x antibody anti-SSEA-1 (mouse monoclonal IgM
as ascites; Solter and Knowles, Proc. Natl. Acad. Sci. USA,
vol. 75, pp. 5565-5569 (1978)) was provided by Dr. D. Solter

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(Philadelphia). Anti-sialyl Lex antibody CSLEX1 (mouse
monoclonal IgM, HPLC purified; Fukushima et al., _Cancer Res._,
vol. 44, pp. 5279-5285 (1984)) and anti-sialyl Lea antibody
CSLEA1 (mouse monoclonal IgG3, ammonium sulfate precipitate;

5   Galton et al., in _Antibodies: Protective, Destructive, and
Regulatory Role. Ninth Int. Convoc. Immuno., Amherst, NY, pp.
117-125, Karger, Basel (1985); and Chia et al., _Cancer Res._,
vol. 45, pp. 435-437 (1985)) were provided by Dr. P. Terasaki
(Los Angeles). Anti-H and anti-Lea[a] antibodies (both mouse

10   monoclonal IgM, antigen affinity purified) were purchased from
Chembiomed Ltd. (Edmonton). Anti-ELAM-1 antibody BB11
(Benjamin et al., _Biochem. Biophys. Res. Commun._, vol. 171,
pp. 348-353 (1990)) was the gift of Dr. Roy Lobb (Biogen,
Inc., Cambridge, MA). IgG2b, anti-CD11b, anti-CD19, and

15   anti-CD33 antibodies were purchased from Coulter Corp
(Hialeah, FLA). Anti-VCAM antibody 4B9 (Carlos and Harlan,
Immunol. Rev., vol. 114, pp. 1-24 (1990)) was provided by Dr.
John Harlan (University of Washington, Seattle). Anti-ICAM1
antibody 84H10 (Makgoba et al., _Nature_, vol. 331, pp. 86-88

20   (1988)) was purchased from AMAC, Inc. Anti-HLA class I
antibody W6/32 (Parham et al., _J. Immunol._, vol. 123, pp. 342-
349 (1979)) was purchased from SeraLab, Inc. (U.K).

Transfection, ^{51}Cr-Labeling, and Harvesting of COS-1 cells

         COS-1 cells were transfected with various plasmids using

25   the DEAE-dextran procedure (Davis et al., _Basic Methods in
Molecular Biology, Elsevier Publishing Co., NY, NY, (1986)) as
previously described (Larsen et al., _Proc. Nat. Acad. Sci._
USA, vol. 86, pp. 8227-8231 (1989)). Approximately 72 hours
after transfection, cells (approximately 1 x 10^6) were labeled

30   with ^{51}Cr by incubating them for 3-4 hours with 10 μCi/ml of

Na^{51} CrO$_4$ (NEN). Labeled or unlabeled, transfected COS-1 cells
were harvested for HUVEC binding assays, or for flow cytometry
analyses, by washing the cell monolayers with calcium,
magnesium free PBS (CMF-PBS) containing 2 mM EDTA, and then
5. incubating the washed monolayers with CMF-PBS/2 mM EDTA.
Detached cells were washed once by centrifugation through
CMF-PBS/2 mM EDTA, and then resuspended in buffers compatible
with flow cytometry or binding analyses.

Construction and radiolabeling of stably transfected CHO cell
10 lines

        CHO Ade-C cells (Oates and Patterson, Somatic Cell
Genet., vol. 3, pp. 561-577 (1977); and Van Keuren et al., Am.
J. Hum. Genet., vol. 38, pp. 793-784 (1986)) were transfected
(Chen and Okayama, Mol. Cel. Biol., vol. 7, pp. 2745-2752
15 (1987)) with Xhol-linearized pCDM7-α(1,3/1,4)FT
(Kukowska-Latallo et al., Genes Dev., vol. 4, pp. 1288-1303
(1990)), or with Xhol-linearized pCDM7 (Larsen et al., Proc.
Natl. Acad. Sci. USA, vol. 86, pp. 8227-8231 (1989)), each
co-precipitated in a 10-fold molar excess over
20 EcoRl-linearized pSV2-Neo (Southern and Berg, J. Mol. Appl.
Genet., vol. 1, pp. 327-341 (1982)). Approximately 26% of the
G418-resistant transfectants generated with pCDM7-α(1,3/1,4)FT
stained positively with an anti-Lex antibody (anti-SSEA-1,
Solter and Knowles, Proc. Natl. Acad. Sci. USA, vol. 75, pp.
25 5565-5569 (1978)). A single, clonal, SSEA-1 positive cell
line (CHO-FT) was derived from this population. The
G418-resistant transfectants generated with pCDM7 were
maintained as an uncloned, pooled population (CHO-V). Cell
extracts prepared from CHO-FT contained substantial amounts of
30 α(1,3) fucosyltransferase activity when assayed with the

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acceptor N-acetyllactosamine (Kukowska-Latallo et al., <u>Genes</u>
<u>Dev.</u>, vol. 4, pp. 1288-1303 (1990)), whereas extracts prepared
from the parental cell line and from the CHO-V cells contained
no detectable fucosyltransferase activity. CHO-FT or CHO-V (6
5   x 10^6) were labeled with ^{51}Cr by incubating them for 5-6 hours
in 25 μCi/ml of Na^{51}Cr. Labelled cells were then harvested
using the procedure described above for COS-1 cells.

HUVEC Adhesion Assays

Two methods were used for quantitating heterotypic
10 adhesion to HUVEC monolayers.

Method I

Method I was used for analyses with transfected cells,
and is a conventional HUVEC adhesion assay utilizing ^{51}Cr
labelled cells incubated at 37°C. HUVEC in 96-well plates
15 were placed in growth media without growth factors, with or
without 20 ng/ml TNFα (Genentech), for 4 to 6 hours prior to
binding assays. In experiments where inhibitory antibodies
were used, antibodies were added in 50 μl PBS containing 100
μg per ml each of Ca and Mg (PBS/Ca/Mg), 0.5% human serum
20 albumin (endotoxin-free, Cutter), and incubated with HUVEC at
4°C for 1 hour. Antibodies were added to a final
concentration of 1 μg/ml, except the anti-HLA class I
antibody, which ascites used at a 1:1000 dilution. These
represent saturating concentrations, as determined by
25 radioimmunoassay. After treating with antibodies, HUVEC
monolayers were washed three times with PBS/Ca/Mg. Cells to
be tested for adhesion were harvested as detailed above,
resuspended in PBS/Ca/Mg, and held at 4°C for the shortest

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time possible until used in the assay. Cells (3×10^5
neutrophils, 1×10^5 COS-1 cells, 3×10^5 CHO cells) were
added to HUVEC monolayers in 100 μl of PBS/Ca/Mg, and
incubated at 37°C. Microscopic titration was used to
5 determine the numbers of cells added of each type (sufficient
to just allow cells to form an essentially confluent monolayer
overlying the HUVEC monolayer, providing the majority of added
cells an opportunity to interact with the underlying
endothelium without multilayering). Cells were allowed to
10 adhere for various times (10 to 25 minutes, see Figure
legends). Unbound cells were removed by exchanging the wells
three times with 150 μl of PBS/Ca/Mg. Each well was then
counted in a gamma counter, and the number of bound cells was
calculated based upon a previous determination of the number
15 of cpms incorporated per radiolabeled cell.

Method II

     Method II maximizes carbohydrate-dependent adhesion by
adopting principles used to measure the lectin-dependent
adhesion of lymphocytes to frozen sections of lymph nodes
20 (Stoolman et al., Blood, vol. 70, pp. 1842-1850 (1987)). This
method was used to determine ELAM-1-dependent adhesion of the
HL-60 myeloid cell lines. HUVEC were plated in 96-well tissue
culture plates; 24 hours later, the confluent HUVEC monolayers
were incubated with 20 ng/ml of TNFα for 4-6 hours prior to
25 initiating binding assays. HL-60 cells were washed in MEM+
(minimal essential medium buffered to pH 7.3 with Tricine, 40
mM, and supplemented with 1 mg/ml bovine serum albumin)
immediately prior to fixation. Immediately prior to the
binding assay, cells in suspension were fixed in freshly
30 prepared paraformaldehyde (0.5% paraformaldehyde in 0.15 M

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cacodylate buffer, pH 7.4; 20 minutes, 4°C), washed
extensively, and resuspended at 10^6 cells/ml in MEM+. In some
experiments, the TNFα-treated HUVEC monolayers were
preincubated at 7-10°C with 50 μl of either IgG2b or BB11

5   monoclonal antibodies at a concentration of 20 μ/ml. Fixed
cells (10^5 cells in 50 μl) were generally added directly to
the plates after 15-30 minutes of preincubation (washing the
HUVEC monolayers free of unbound antibody prior to instituting
the binding assays had no effect on the level of inhibition,

10   data not shown). Added cells were allowed to settle and
interact with the HUVEC for 45 minutes at 7-10°C prior to
quantitation of adhesion.

       Unattached cells were removed by aspirating the cell
suspensions and gently washing the monolayers three times with

15   200 μl of MEM+ at room temperature. The wash solution was
applied with an 8-channel multipipettor to the upper wall of
the wells while maintaining the plates at a 30° angle. The
solutions were then drawn off the lower wall without
contacting the monolayer. The number of cells recovered from

20   each well was determined by counting the pooled washes on a
Coulter ZBI cell counter. The number of bound cells
represents the difference between the number of cells applied
to each well and the number recovered from the well.

Flow Cytometry

25   Transfected COS-1 cells, or CHO transfectants, were
subjected to flow cytometry analysis with mouse monoclonal IgM
antibodies directed against carbohydrate epitopes, using
procedures described previously (Ernst et al., J. Biol. Chem.,
vol. 264, pp. 3436-3447 (1989); and Kukowska-Latallo et al.,

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Genes Dev., vol. 4, pp. 1288-1303 (1990)). Cells were stained
with saturating amounts of anti-SSEA-1 monoclonal antibody
(1:1000 dilution of ascites), anti-Lewis a monoclonal antibody
(10 μg/ml), anti-H antibody (10 μg/ml), or anti-CSLEX1 (10

5   μg/ml). Cells were then stained with fluorescein-conjugated
goat anti-mouse IgM (Sigma; 40 μg/ml) and subjected to
analysis by flow cytometry on a FACScan (Becton Dickinson,
Mountain View, CA), as described previously (Ernst et al., J.
Biol. Chem., vol. 264, pp. 3436-3447 (1989); and Larsen et

10 al., Proc. Natl. Acad. Sci. USA, vol. 86, pp. 8227-8231
(1989)). Cell staining was measured in arbitrary fluorescent
intensity units, and was displayed on a four decade log scale.
Analyses with the IgG3 class mouse anti-CSLEA1 antibody (10.8
μg/ml), or the pooled mouse IgG control antibody (MsIg,

15 Coulter, 10 μg/ml) were performed in an identical manner,
except that a FITC-conjugated goat antimouse IgG antibody
(Sigma, 40 μg/ml) was used.

Flow cytometry-based indirect immunofluorescence analyses
of HL-60 cells, were conducted in microtiter plates. Both

20 primary and secondary antibodies were used at saturating
concentrations. Antigen specific mouse anti-human monoclonal
antibodies (50 μL) were combined with cells (2.5×10^5 in 50
μL) and incubated at 4°C for 30 minutes in PBS+ (phosphate
buffered saline supplemented with 1% fetal bovine serum and

25 0.05% Na azide). Cells were washed twice in PBS+ (200 μL),
resuspended and combined with 50 μL of FITC-labeled goat
anti-mouse IgG (Fab' 2 fragments, Cappel Laboratories, West
Chester, PA) for an additional 30 minutes at 4°C. After three
washes in PBS+, pellets were resuspended and combined with 200

30 μL of 1-2% paraformaldehyde prior to cytometric analysis.
Fluorescence quantitation was performed on a Coulter Epics V

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Flow Cytometer equipped with a three-decade scale.

<u>Results</u>

<u>ELAM-1 Dependent Cell Adhesion Correlates with sLex Expression
in Variants of the HL-60 Cell Line</u>

5   The ELAM-1 receptor mediates the adhesion of neutrophils,
monocytes, and the related cell lines HL-60 and U937 to
cytokine-stimulated human umbilical vein endothelial cells
(HUVEC) (Bevilacqua et al., <u>Proc. Natl. Acad. Sci. USA</u>, vol.
84, pp. 9238-9242 (1987); and Bevilacqua et al., <u>Science</u>, vol.
10   243, pp. 1160-1165 (1989)). The surfaces of these leukocytic
cells are unusually rich in fucosylated derivatives of neutral
and $\alpha(2,3)$sialylated polylactosamine ($[Gal\beta1,4GlcNAc)_n]$)
moieties (Fukuda et al. <u>J. Biol. Chem.</u>, vol. 259, pp. 10925-
10935 (1984); Fukuda et al., <u>J. Biol. Chem.</u>, vol. 260, pp.
15   1067-1082 (1985); and Spooncer et al., <u>J. Biol. Chem.</u>, vol.
259, pp. 4792-4801 (1984)). The structurally and
biosynthetically related members of this group include the
sialyl Lewis X tetrasaccharide
(NeuAc$\alpha2\rightarrow3$Gal$\beta1\rightarrow4$(Fuc$\alpha1\rightarrow3$)GlcNAc, sLex; Fukushima et al.,
20   <u>Cancer Res.</u>, vol. 44, pp. 5279-5285 (1984); Figure 1a), its
non-sialylated trisaccharide analogue Lewis X
(Gal$\beta1\rightarrow4$(Fuc$\alpha1\rightarrow3$)GlcNAc, Lex or SSEA-1; Gooi et al., <u>Nature</u>,
vol. 292, pp. 156-158 (1981); Figure 1A), and related neutral
and $\alpha(2,3)$sialic acid-substituted structures containing single
25   (VIM-2, Macher et al., <u>J. Biol. Chem.</u>, vol. 263, pp. 10186-
10191 (1988)) or multiple (FH4 and FH5, Fukushi et al., <u>J.
Biol. Chem.</u>, vol. 259, pp. 4681-4685 (1984); FH6, Fukushi et
al., <u>Cancer Res.</u>, vol. 45, pp. 3711-3717 (1985)) internal
$\alpha(1,3)$-linked fucose residues. By contrast, these

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oligosaccharide determinants are generally not detected on circulating cells which lack the ligand(s) for ELAM-1 (Fox et al., Cancer Res., vol. 43, pp. 669-678 (1983); Fukushima et al., Cancer Res., vol. 44, pp. 5279-5285 (1984); and Fukushi
5 et al., Cancer Res., vol. 45, 3711-3717 (1985). These observations prompted us to measure the expression of sLex and Lex epitomes on two variants of the HL-60 line showing markedly different levels of ELAM-1-dependent adhesion to TNFα-treated HUVEC.

10   Figure 2 depicts a series of experiments with two spontaneously arising variants of the HL-60 line. Both cell lines expressed the myeloid differentiation antigens CD11b, CD19 and CD33 (data not shown). Using an assay that maximizes carbohydrate-dependent cell adhesion (Stoolman et al., Blood,
15 vol. 70, 1842-1850 (1987) and Experimental Procedures), HL-60 line B cells bound avidly to TNFα-treated HUVEC, with 55% of the added cells attached in the presence of the control antibody IgG2b. This adhesion was largely ELAM-1-dependent since only 11% of the cells adhered in the presence of the
20 anti-ELAM-1 antibody BB11 (Benjamin et al., Biochem. Biophys. Res. Commun., vol. 171, pp. 348-353 (1990)). By contrast, the line designated HL-60 A exhibited a lower absolute level of adhesion (mean of 16%) which was not significantly reduced by treatment of the HUVEC with BB11 (14%). Thus HL-60 A showed
25 virtually no ELAM-1-dependent adhesion under conditions that optimized detection of this interaction on HUVEC monolayers.

Flow cytometer analysis revealed markedly different levels of the sLex and Lex determinants at the cell surface of HL-60 A and HL-60 B (Figure 2b). Both cell lines expressed
30 the Lex epitope as has been previously described for the HL-60

-37-

cell line (Symington et al., J. Immun., vol. 134, pp. 2498-
2506 (1985)). By contrast, the sLex epitope was detected on
the adhesion competent HL-60 line B cells exclusively. The
resistance of the ELAM-1 ligand(s) to paraformaldehyde
5 fixation and the persistence of its functional activity at
reduced temperature mirror the behavior of the endogenous,
sialylated ligand for LEC-CAM1 (Stoolman, Cell, vol. 56, pp.
907-910 (1989); and Yednock and Rosen, Adv. Immunol., vol. 44,
pp. 313-378 (1989)), and thus are consistent with the
10 hypothesis that the CRD of ELAM-1 interacts with an
oligosaccharide on the surface of HL-60 cells. Moreover, the
coordinate loss of both ELAM-1-dependent adhesion and the sLex
determinant suggested that the ligand(s) belongs to the family
of sialylated, fucosylated polylactosaminoglycans expressed at
15 high levels on myeloid cells.

ELAM-1 Dependent Cell Adhesion of COS-1 Cells Determined by
Transfection of a Cloned Fucosyltransferase cDNA

The profound differences in sLex expression observed in
the HL-60 variants may have resulted from differences in the
20 activities of specific fucosyltransferases (Figure 1a), by
analogy to the Chinese hamster ovary (CHO) glycosylation
mutants Lec11 and Lec12 (Howard et al., J. Biol. Chem., vol.
262, pp. 16830-16837 (1987)). The LEC11 mutant exhibits de
novo expression of a specific $\alpha(1,3)$fucosyltransferase, termed
25 Fuc-TI, that determines the synthesis of surface-localized
neutral and $\alpha(2,3)$sialylated polylactosaminoglycans
substituted with $\alpha(1,3)$-linked fucose residues (Figure 1a).
By contrast, the Lec12 mutant expresses a distinct
$\alpha(1,3)$fucosyltransferase, Fuc-TII, that determines surface
30 display of neutral, $\alpha(1,3)$-fucosylated polylactosaminoglycans,

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but not the α(2,3)sialylated analogues (Figure 1a). The
penultimate step in the biosynthesis of these structures,
which include the sialyl Lex moiety, is thought to be
catalyzed by a widely distributed sialyltransferase that
5   attaches sialic acid in an α(2,3) linkage to terminal
galactose residues in the polylactosamine substrate (Weinstein
et al., _J. Biol. Chem._, vol. 257, pp. 13845-13853 (1982)).
These sialylated molecules can then serve as acceptors for
some (Holmes et al., _J. Biol. Chem._, vol. 261, pp. 3737-3743
10   (1986); and Howard et al., _J. Biol. Chem._, vol. 262, pp.
16830-16837 (1987)), but not all (Howard et al., _J. Biol._
Chem., vol. 262, pp. 16830-16837 (1987))
α(1,3)fucosyltransferases. These enzymes may add fucose
residues in an α(1,3)-linkage to the terminal GlcNAc residue,
15   thus forming the sLex structure, and/or to one or more GlcNAc
residues within internal lactosamine units (Holmes et al., _J._
Biol. Chem., vol. 261, pp. 3737-3743 (1986); and Howard et.
al., _J. Biol. Chem._, vol. 262, pp. 16830-16837 (1987)).
Likewise, α(1,3)fucosyltransferases can generate the Lex
20   moiety and its polyfucosylated analogues from neutral
polylactosamine precursors. These neutral fucosylated
molecules are not, however, substrates for any known
α(2,3)sialyltransferases. These considerations suggest a
critical, regulatory role for expression of specific
25   α(1,3)fucosyltransferases in the biosynthesis of the family of
sialylated, fucosylated lactosaminoglycans that represent
putative ELAM-1 ligands.

To investigate this possibility, and to test the roles of
these molecules in ELAM-1-dependent cell adhesion, their _de_
30   _novo_ surface expression on mammalian host cells was effected,
via modification of their glycosylation phenotypes with

transfected α(1,3)fucosyltransferase cDNAs or gene segments
(pCDM7-α(1,3/1,4)FT, Kukowska-Latallo et al. <u>Genes Dev.</u>, vol.
4, pp. 1288-1303 (1990); and pCDNA1-α(1,3)FT, Lowe et al.,
unpublished). Non-myeloid hosts were chosen for these
5 experiments in order to isolate the role of the
oligosaccharide molecules in ELAM-1-dependent adhesion from
other myeloid-specific molecules known to participate in
leukocyte-endothelial cell interactions (Springer, <u>Nature</u>,
vol. 346, pp. 425-434 (1990)). COS-1 cells were used for the
10 first of these experiments since these cells do not express
detectable α(1,3)fucosyltransferase activity nor cell surface
oligosaccharides that contain the cognate α(1,3)fucose
linkages (Kukowska-Latallo et al., <u>Genes Dev.</u>, vol. 4, pp.
1288-1303 (1990)). Moreover, COS-1 cells do not exhibit
15 ELAM-1-dependent adhesive properties. They do, however,
express the oligosaccharide substrates necessary for
α(1,3)fucosyltransferase-dependent biosynthesis of the family
of oligosaccharides represented by the Lex and sialyl Lex
molecules (Fukuda et al., <u>J. Biol. Chem.</u>, vol. 263, pp. 5314-
20 5318 (1988); and Kukowska-Latallo et al., <u>Genes Dev.</u>, vol. 4,
pp. 1288-1303 (1990)).

As shown in Figure 3a, 26-31% of the population of COS-1
cells transfected with a plasmid encoding an
α(1,3/1,4)fucosyltransferase (pCDM7-α(1,3/1,4)FT,
25 Kukowska-Latallo el al. <u>Genes Dev.</u>, vol. 4, pp. 1288-1303
(1990)) express sLex and Lex molecules (Figure 3a). As
expected from the properties of the enzyme encoded by
pCDM7-α(1,3/1,4)FT (Prieels et al., <u>J. Biol. Chem.</u>, vol. 256,
pp. 10456-10463 (1981); Palcic et al., <u>Carbohyd. Res.</u>, vol.
30 190, pp. 1-11 (1989); and Kukowska-Latallo et al., <u>Genes Dev.</u>,
vol. 4, pp. 1288-1303 (1990)), the sLea and Lea determinants

-40-

are also expressed (Figures 1b and 3a). By contrast, COS-1
cells transfected with pCDNA1-α(1,3)FT, which
encodes a distinct α(1,3)fucosyltransferase (Lowe et al.,
unpublished), express new Lex determinants exclusively (Figure
5 3a). Cells transfected with the control vectors (pCDM7 and
pCDNA1), as well as with vectors encoding two other mammalian
glycosyltransferases (pCDM7-αGT, Larsen et al., Proc. Natl.
Acad. Sci. USA, vol. 86, pp. 8227-8231 (1989); and pH3.4,
Rajan et al., J. Biol. Chem., vol. 264, pp. 11158-11167
10 (1989)) remained negative for all four surface carbohydrate
determinants (Figure 3a and data not shown). Using
^{51}Cr-labeled cells, only pCDM7-α(1,3/1,4)FT transfectants,
expressing the four new fucosylated oligosaccharide
determinants, exhibited adhesion to TNFα-treated HUVEC (Figure
15 3b). This binding was substantially inhibited with the
anti-ELAM-1 antibody BB11, but not with its isotype control
antibody IgG2b, nor with antibodies that detect other
polypeptides expressed on TNFα-treated HUVEC (VCAM1, ICAM1,
and HLA class I determinants, Figure 3c).

20   In these experiments, approximately 7% of the pCDM7-α
(1,3/1,4)FT transfectants adhered to TNFα-treated endothelium,
whereas 35% of normal neutrophils adhered under the same
conditions (Figure 3b, right). This apparent difference in
relative adhesion "activity" can be accounted for by the fact
25 that only a minority (26-31%) of the radiolabeled COS-1 cells
actually express new cell surface oligosaccharide
determinants, and by the fact that ELAM-1 interactions
represent only one of several mechanisms responsible for
adhesion of neutrophils to cytokine-stimulated endothelium
30 (Luscinska et al., J. Immunol., vol. 142, pp. 2257-2263
(1989)). Indeed, pretreatment of the TNFα-activated HUVEC in

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this experiment incompletely blocked neutrophil adhesion,
reducing it to approximately 17% (data not shown). These
considerations indicate that ELAM-1-dependent adhesion of the
two cell types is of a substantially similar magnitude.

5   The neutral and sialylated Lea isomers (Figure 1b)
displayed by the pCDM7-α(1,3/1,4)FT transfected,
adhesion-competent COS-1 cells are generally absent from blood
cells of the myeloid lineage (Fukuda et al., J. Biol. Chem.,
vol. 259, pp. 10925-10935 (1984); Fukuda et al., J. Biol.
10 Chem., vol. 260, pp. 1067-1082 (1985); Spooncer et al., J.
Biol. Chem., vol. 259, pp. 4792-4801 (1984); and Dunstan, Br.
J. Haemat., vol. 62, pp. 301-309 (1986)) and thus would not
normally participate in leukocyte-ELAM-1 adhesive
interactions. It is therefore concluded that the
15 ELAM-1-dependent adherence of cells transfected with
pCDM7-α(1,3/1,4)FT is mediated by the surface-localized sialyl
Lex tetrasaccharide molecule, and/or its alternately and poly
α(1,3)fucosylated analogues, but not by the neutral family of
molecules represented by the Lex determinant.

20 Expression of a Cloned Fucosyltransferase cDNA in
Stably-transfected CHO Cells Determines ELAM-1 Dependent Cell
Adhesion and sLex Expression

         To isolate and further confirm the contribution of the
sialylated Lex-type oligosaccharide molecules to
25 ELAM-1-dependent binding, we generated a transfected mammalian
cell line (CHO-FT) that expresses surface-localized sLex and
Lex determinants, but not the Lea or sLea determinants. The
parental CHO cell line Ade⁻ C (Oates et al., Somatic Cell
Genet., vol. 3, pp. 561-577 (1977); and Van Keuren el al., Am.

J. Hum. Genet., vol. 38, pp. 793-804 (1986)) chosen for these
experiments expresses no detectable α(1,3)fucosyltransferase
activity (vide supra) nor the corresponding Lex and sialyl Lex
molecules (Smith et al., _J. Biol. Chem._, vol. 265, pp. 6225-
5   6234 (1990)). It does, however, express polylactosaminoglycan
precursors for these molecules, but not for the Lea precursors
(Smith et al., _J. Biol. Chem._, vol. 265, pp. 6225-6234
(1990)). Expression of stably-transfected pCDM7-α(1,3/1,4)FT
in this host determines surface display of substantial amounts
10 of the sLex determinant, and to a lesser extent the Lex
determinant, but not the Lea isomers (Figure 4a).

Each CHO transfectant line was assessed for adhesion to
untreated and TNFα-treated HUVEC (Figures 4b and 4c). Cells
were allowed to adhere for between 5 and 25 minutes, before
15 washing off unbound cells. CHO-FT cells exhibited marked
adhesion to TNFα-treated HUVEC, but not to untreated
endothelium (Figure 4b). Adhesion of CHO-FT cells was well-
established within 5 minutes, peaked at 10 minutes, and was
resistant to repeated and vigorous washing (vide supra). By
20 contrast, control transfectants (CHO-V), that do not express
significant amounts of the Lex or sialyl Lex molecules (Figure
4a), did not exhibit meaningful binding to either TNFα-treated
or untreated HUVEC (Figure 4b) at early time points, and
minimal, non-specific adhesion at later ones. This adhesion
25 was ELAM-1-dependent since it was inhibited by pretreatment of
the HUVEC with the BB11 anti-ELAM-1 antibody, but not by the
control antibody IgG2b, nor with antibodies directed against
other adhesion receptors (VCAM1 and ICAM1), or to HLA class I
determinants (Figure 4c and data not shown). The very rapid
30 onset of the ELAM-1-dependent adhesion to vascular endothelium
exhibited by the CHO-FT cells is physiologically consistent

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with the speed of the analogous interaction between
neutrophils and ELAM-1, that arrests these latter cells as
they pass over activated endothelium.

5   Obviously, numerous modifications and variations of the
present invention are possible in light of the above
teachings. It is therefore to be understood that, within the
scope of the appended claims, the invention may be practiced
otherwise than as specifically described herein.

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Claims:

    1.   A compound of formula (I):

$$\text{NeuAc}\alpha 2 \rightarrow 3\text{Gal}\beta 1 \rightarrow 4(\text{Fuc}\alpha 1 \rightarrow 3)\text{GlcNAc}\alpha 1 \rightarrow R \qquad (I)$$
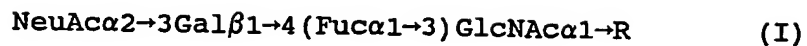
wherein R is OH, a protected hydroxy group, a C_{1-12} alkyl
5   group, $-O-(CH_2)_n-CO_2R''$ (wherein R'' is C_{1-4} alkyl and n is 2 to
12), $-O-(CH_2)_n\text{CONHNH}_2$ (n is 2 to 12), $-O-(CH_2)_n\text{CON}_3$ (n is 2 to
12), a lipid, a glycolipid, a glycoconjugate of a
serine/threonine-linked oligosaccharide or a protein or a
polypeptide, a glycoconjugate of a free oligosaccharide
10 derived by chemical or enzymatic hydrolysis of
serine/threonine-linkage to a protein or polypeptide, a
glycoconjugate of an asparagine-linked oligosaccharide on a
protein or polypeptide, or a glycoconjugate of a free
oligosaccharide derived by enzymatic or chemical hydrolysis
15 from an asparagine linkage to a protein or polypeptide, a
moiety derived from a drug, a moiety derived from a
fluorescent or chemiluminescent label, or a group of the
formula -X-R', wherein X is a bivalent linking group and R' is
a moiety derived from a drug or a moiety derived from a
20 fluorescent or chemiluminescent label.

    2. A method for treating a disease mediated by the
elaboration of ELAM-1 on endothelial cells, comprising
administering a therapeutically effective amount of a compound
of Claim 1, to a patient in need thereof.

25   3. The method of Claim 2, wherein said disease is
selected from the group consisting of adult respiratory

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distress syndrome, vasculitis, and myocardial infarction.

4.   A transfected cell, which contains a heterologous
sequence of DNA which encodes an enzyme which exhibits the
activity of converting a substrate produced by the cell to the
5 sialyl Lewis X determinant.

5.   The cell of Claim 4, wherein said enzyme is the
fucosyltransferase encoded by pCDM7-α(1,3/1,4FT).

6.   A process for preparing a transfected cell,
comprising:

10   (i)   transfecting a cell with a heterologous sequence of
DNA encoding an enzyme having the activity of converting a
substrate produced by said cell to the sialyl Lewis X
determinant.

7.   The process of Claim 6, wherein said enzyme is the
15 fucosyltransferase encoded by pCDM7-α(1,3/1,4FT).

8.   A cell, prepared by a process, comprising:

(i)   treating a cell with an enzyme which has the
activity of converting a compound on the surface of said cell
into a compound which contains the sialyl Lewis X determinant.

20   9.   The cell of Claim 8, wherein said enzyme is the
fucosyltransferase encoded by pCDM7-α(1,3/1,4FT).

10.   A process for preparing an oligosaccharide,
comprising:

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(i) treating a cell with an enzyme which has the
activity of converting a compound on the surface of said cell
into a compound which contains the sialyl Lewis X determinant.

11. The process of Claim 10, wherein said enzyme is the
5 fucosyltransferase encoded by pCDM7-α(1,3/1,4FT).

12. In a method for imaging an area of inflammation of
the endothelium by NMR or radionucleotide scanning, the
improvement being the use of (i) a compound of the formula (I)

$$NeuAc\alpha2{\rightarrow}3Gal\beta1{\rightarrow}4(Fuc\alpha1{\rightarrow}3)GlcNAc\alpha1{\rightarrow}R \qquad (I)$$
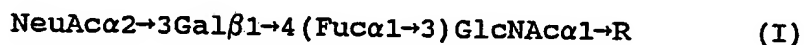
10 wherein R is a moiety derived from a label suitable for said
imaging or a group of the formula -X-R' wherein X represents a
bivalent linking group and R' represents a moiety derived from
a label suitable for said imaging; (ii) a cell which expresses
the sialyl Lewis X determinant and contains a label suitable
15 for said imaging or; (iii) a liposome which carries on its
surface the sialyl Lewis X determinant and comprises a label
suitable for said imaging, to target said label to said area
of inflammation.

13. A liposome, wherein the sialyl Lewis X determinant
20 is present on the surface of said liposome.

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NeuAc $\xrightarrow{\alpha 2,3}$ Gal

$\downarrow \beta 1,4$

$\xleftarrow{\alpha(2,3)\text{SIALYL-T}}$

GlcNAc

$\downarrow$

R

SIALYLATED TYPE II PRECURSOR

$\downarrow$ $\alpha(1,3)\text{FUCOSYL-T}$
e.g. Fuc-TI

NeuAc $\xrightarrow{\alpha 2,3}$ Gal

$\downarrow \beta 1,4$

Fuc $\xrightarrow{\alpha 1,3}$ GlcNAc

$\downarrow$

R

SIALYL LEWIS x

Gal

$\downarrow \beta 1,4$

GlcNAc

$\downarrow$

R

NEUTRAL TYPE II PRECURSOR

$\downarrow$ $\alpha(1,3)\text{FUCOSYL-T}$
e.g. Fuc-TI & Fuc-TII

Gal

$\downarrow \beta 1,4$

Fuc $\xrightarrow{\alpha 1,3}$ GlcNAc

$\downarrow$

R

LEWIS x

FIG. 1A

NeuAc $\xrightarrow{\alpha 2,3}$ Gal

$\downarrow \beta 1,3$

Fuc $\xrightarrow{\alpha 1,4}$ GlcNAc

$\downarrow$

R

SIALYL LEWIS a

Gal

$\downarrow \beta 1,3$

Fuc $\xrightarrow{\alpha 1,4}$ GlcNAc

$\downarrow$

R

LEWIS a

FIG. 1B

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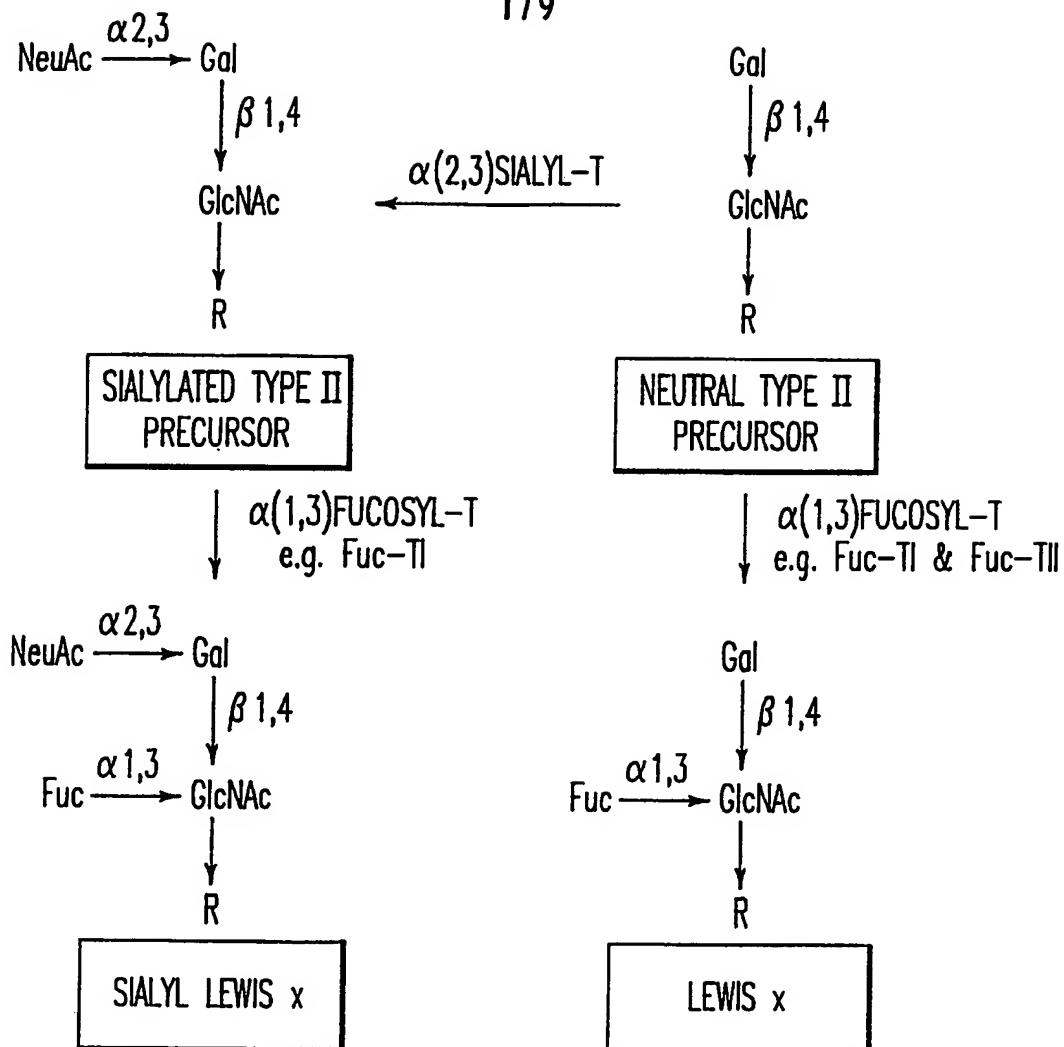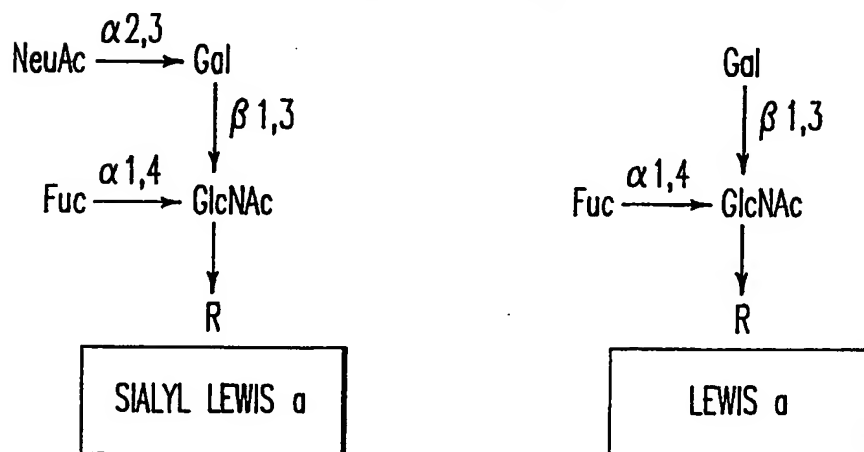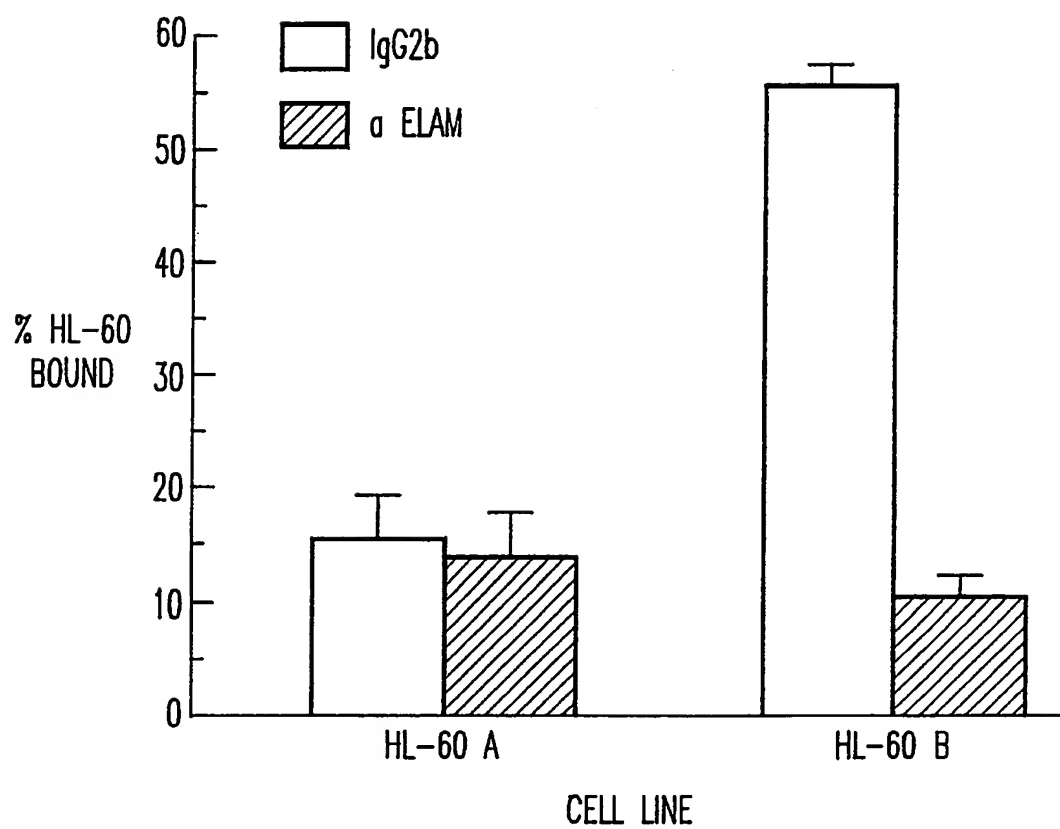
FIG. 2A
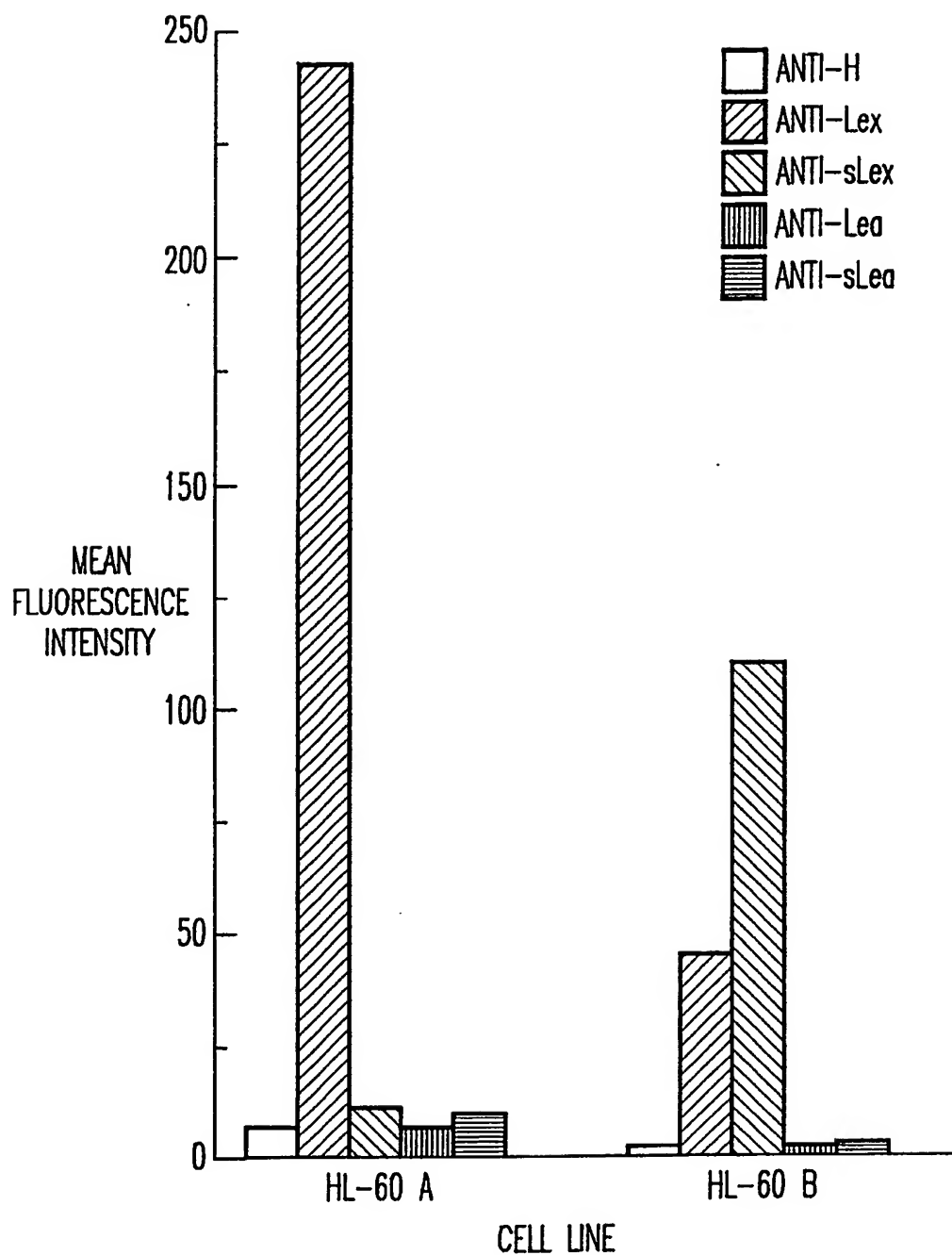
FIG.2B
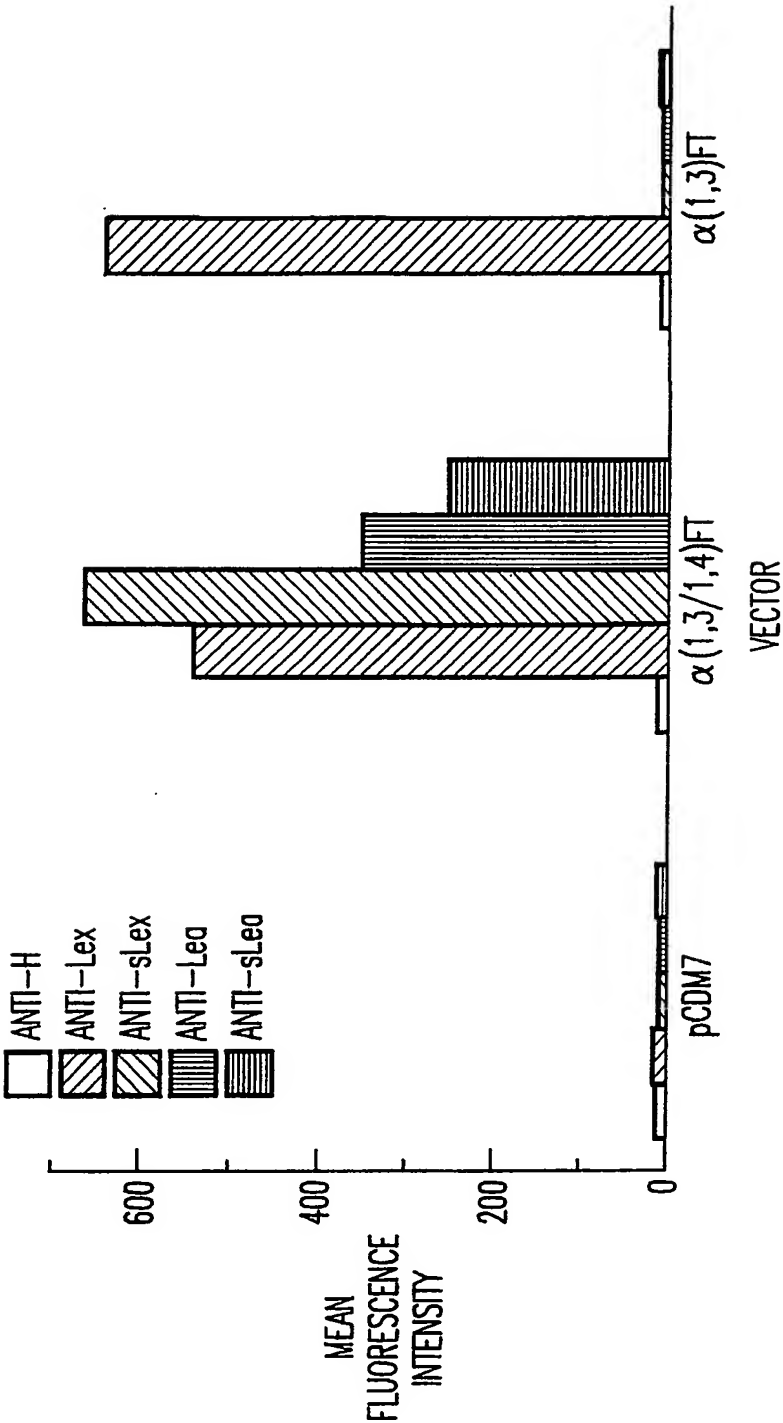
FIG. 3A
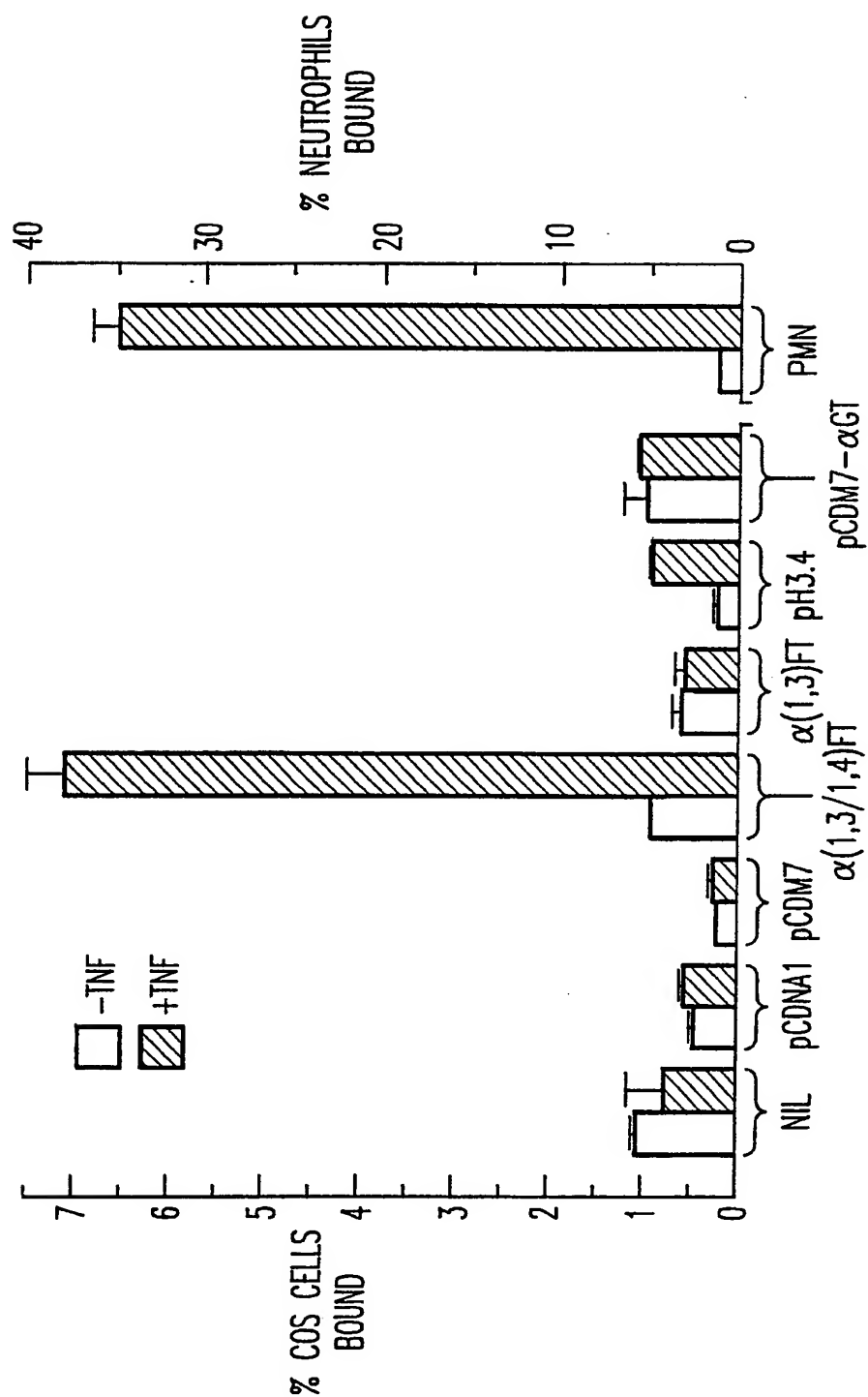
FIG. 3B
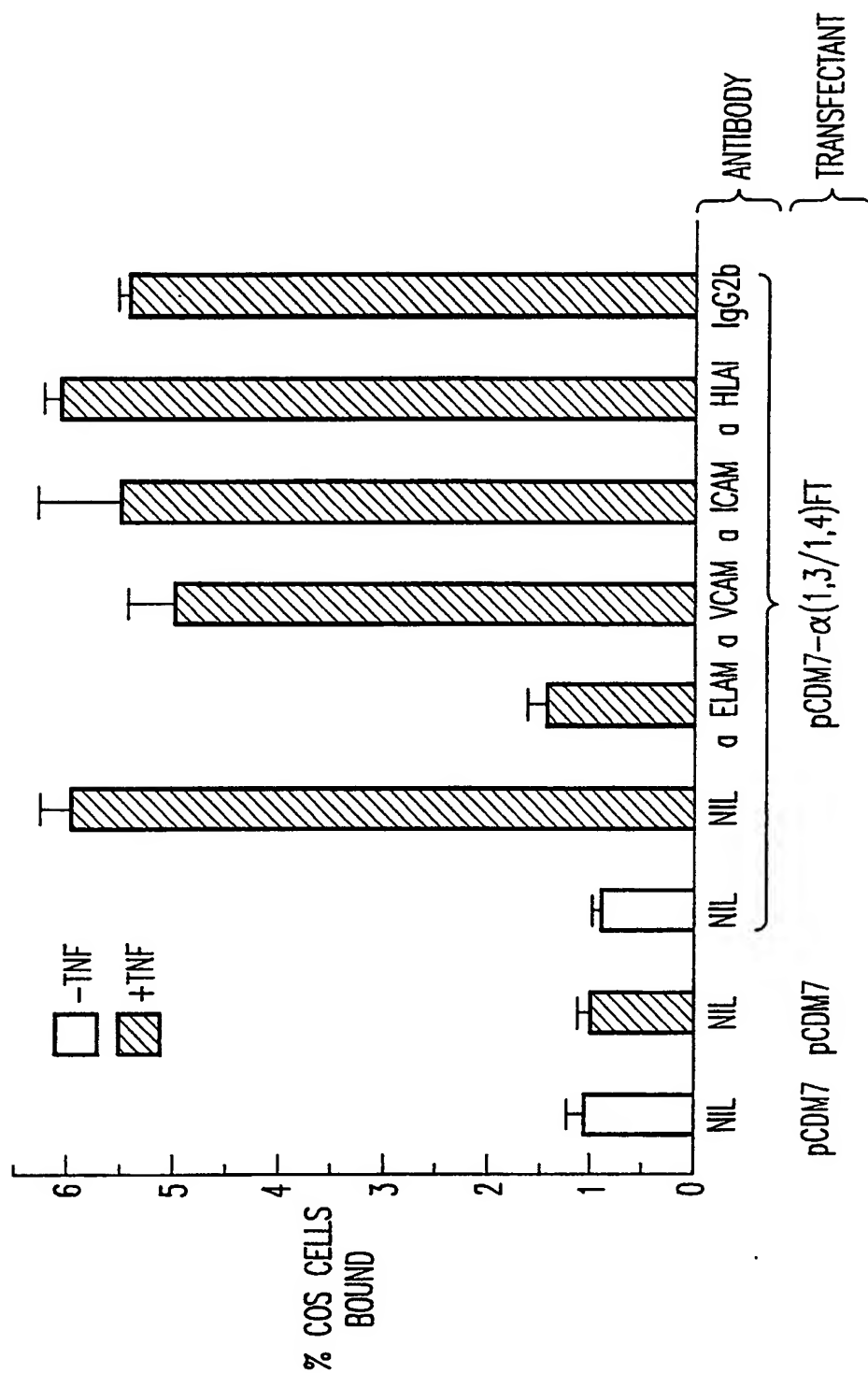
FIG. 3C

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FIG. 4A

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FIG. 4B

FIG. 4C

INTERNATIONAL SEARCH REPORT

I. CLASSIFICATI N OF SUBJECT MATTER (iI several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC(5):A61K 31/73; CO7H 15/04, 5/06; C12N 5/10
U.S. CL.: 435/240.2; 536/53; 514/23, 25, 54

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
U.S.	435/240.2; 536/53; 514/23, 25, 54

Documentation Searched other than Minimum Documentation
to the Extent that such Documents are Included in the Fields Searched [8]

Chemical Abstracts Service: ELAM and receptor# or ligand#

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category [*]	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
X	THE JOURNAL OF BIOLOGICAL CHEMISTRY, Vol. 259, no. 14, issued 25 July 1984, LAMBLIN ET AL., "Primary Structure Determination of Five Sialylated Oligosaccharides Derived from Bronchial Mucus Glycoproteins of Patients Suffering from Cystic Fibrosis", pages 9051-9058, see entire document.	1
X	CANCER RESEARCH, Vol. 44, issued November 1984, FUKUSHIMA ET AL., "Characterization of Sialosylated Lewis X as a New Tumor-Associated Antigen", pages 5279-5285, see entire document.	1
X	EUROPEAN JOURNAL OF BIOCHEMISTRY, Vol. 177, no. 2, issued November 1988, VAN HALBEEK ET AL., "Isolation and Structural Characterization of Low-molecular-mass Monosialyl Oligosaccharides Derived from Respiratory-mucus Glycoproteins of a Patient Suffering from Bronchiectasis", pages 443-460, see entire document.	1

(cont.)

* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
24 January 1992	14 FEB 1992
International Searching Authority	Signature of Authorized Officer
ISA/US	Nancy S. Carson (vsh)

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)		
Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
X	CHEMISTRY AND PHYSICS OF LIPIDS, Vol. 42, issued 1986, HAKOMORI, "Tumor-associated Glycolipid Antigens, Their Metabolism and Organization", pages 209-233, see entire document.	1
X,P	PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES, (USA), Vol. 88, issued February 1991, TIEMEYER ET AL., "Carbohydrate Ligands for Endothelial-leukocyte Adhesion Molecule 1", pages 1138-1142, see entire document.	1-13
X,P	BIOCHEMICAL SOCIETY TRANSACTIONS, Vol. 19, no. 3, issued August 1991, LOWE ET AL., "A Transfected Human Fucosyltransferase cDNA Determines Biosynthesis of Oligosaccharide Ligand(s) for Endothelial-leukocyte Adhesion Molecule 1", pages 649-653, see entire document.	1-13
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A,P	THE JOURNAL OF CELL BIOLOGY, Vol. 115, no. 2, issued October 1991, ZHOU ET AL., "The Selectin GMP-140 Binds to Sialylated, Fucosylated Lactosaminoglycans on both Myeloid and Nonmyeloid Cells", pages 557-564.	1-13
X,P	CELL, Vol. 63, issued 21 December 1990, GOELZ ET AL., "ELFT: A Gene that Directs the Expression of an ELAM-1 Ligand", pages 1349-1356, see entire document.	1-13
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X	PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES (USA), Vol. 87, issued March 1990, HESSION ET AL., "Endothelial Leukocyte Adhesion Molecule 1: Direct Expressions Cloning and Functional Interactions", pages 1673-1677, see entire document.	1-13

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FURTHER INFORMATION C NTINUED FROM THE SEC ND SHEET		
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T	THE JOURNAL OF EXPERIMENTAL MEDICINE, Vol. 174, issued December 1991, **BERG ET AL.**, "The Cutaneous Lymphocyte Antigen In a Skin Lymphocyte Homing Receptor for the Vascular Lectin Endothelial Cell-Leukocyte Adhesion Molecule 1", pages 1461-1466, see entire document.	1-13

V.☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [1]

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1.☐ Claim numbers   . because they relate to subject matter [12] not required to be searched by this Authority, namely:

2.☐ Claim numbers . . . . . , because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out [13], specifically:

3.☐ Claim numbers_____, because they are dependent claims not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI.☐ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [2]

This International Searching Authority found multiple inventions in this international application as follows:

1.☐ As all required additional search fees were timely paid by the applicant, this International search report covers all searchable claims of the international application.

2.☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3.☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4.☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

☐ The additional search fees were accompanied by applicant's protest.

☐ No protest accompanied the payment of additional search fees.

Form PCT/ISA/210 (supplemental sheet (2) (Rev. 11-87)